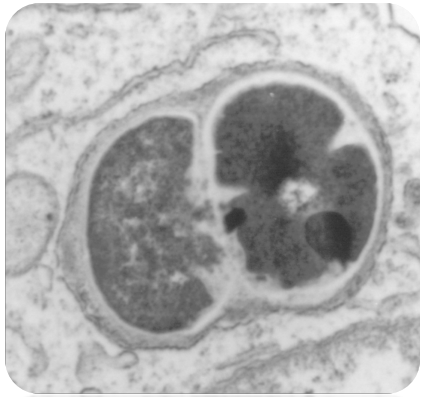


INTRACELLULAR MODELS FOR ANTIMICROBIAL R&D

Françoise Van Bambeke, PharmD, PhD

Pharmacologie cellulaire et moléculaire
Louvain Drug Research Institute
<www.facm.ucl.ac.be>

Disclosures

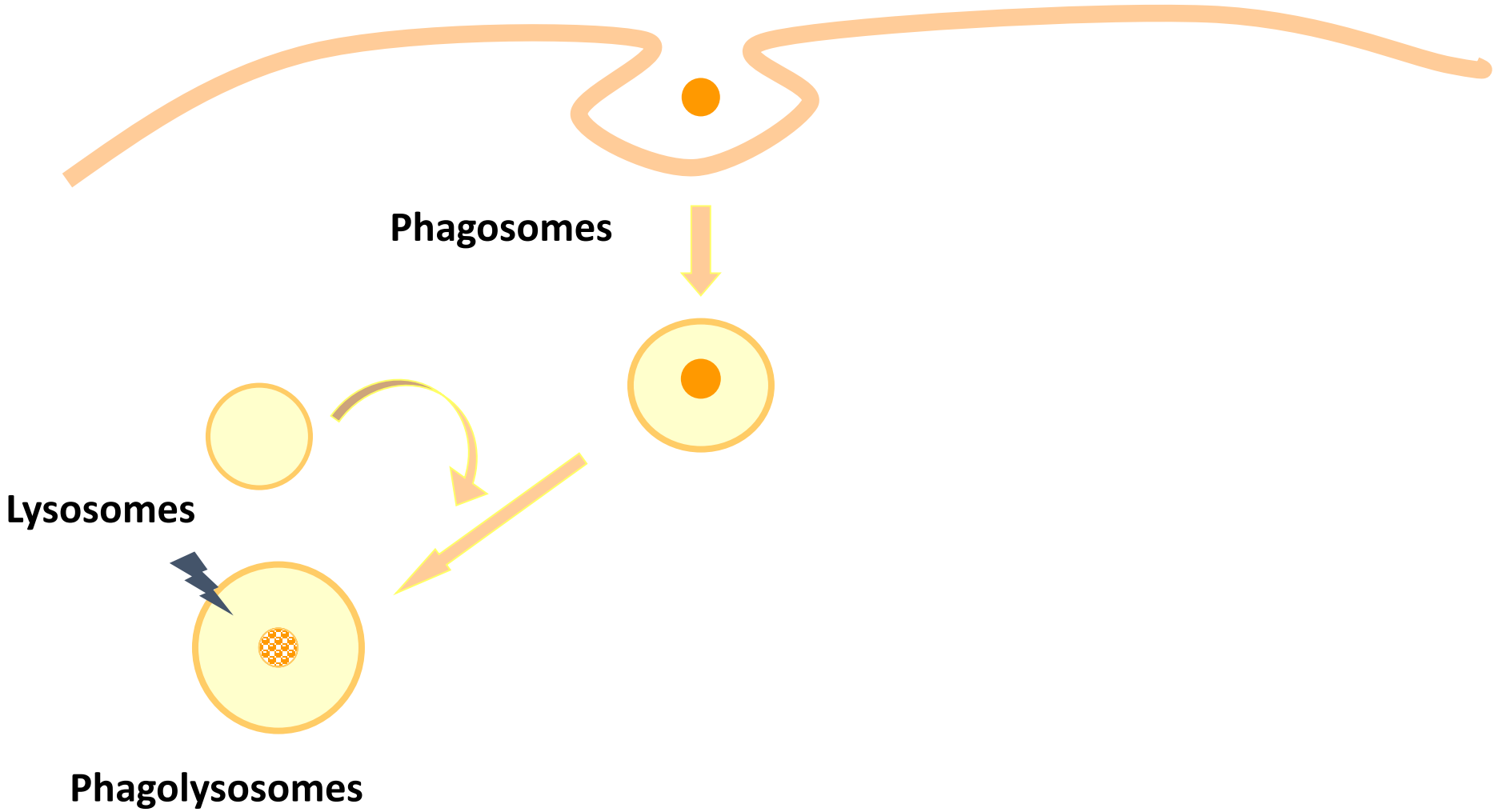
Research grants from many companies over the years for the study of antibiotic PK/PD against intracellular infections

Astra-Zeneca
Bayer
Cempra
Debiopharm
GSK
Melinta
Merlion
Rib-X
Targanta/ The Medicine Company
Thervance
Trius
…

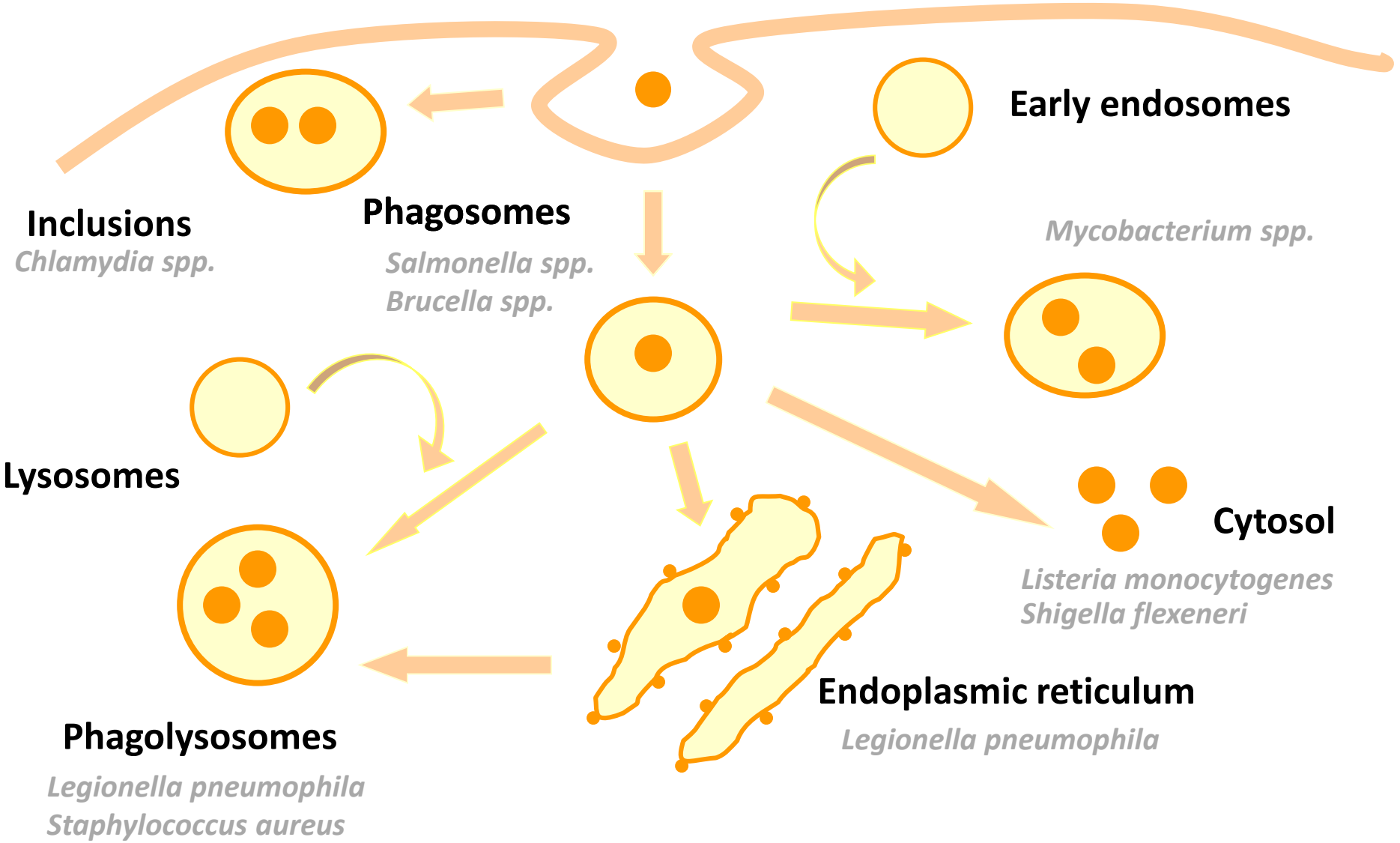
Intracellular models for antimicrobial R&D

- Role of intracellular survival in chronic infections and its contribution to poor response to antibiotics
- In vitro models to study intracellular activity of antibiotics
- Cellular pharmacokinetic (PK) parameters predictive of intracellular potency for antibiotics
- Cellular pharmacodynamic (PD) parameters predictive of intracellular efficacy for antibiotics

Cellular killing of bacteria by host cells

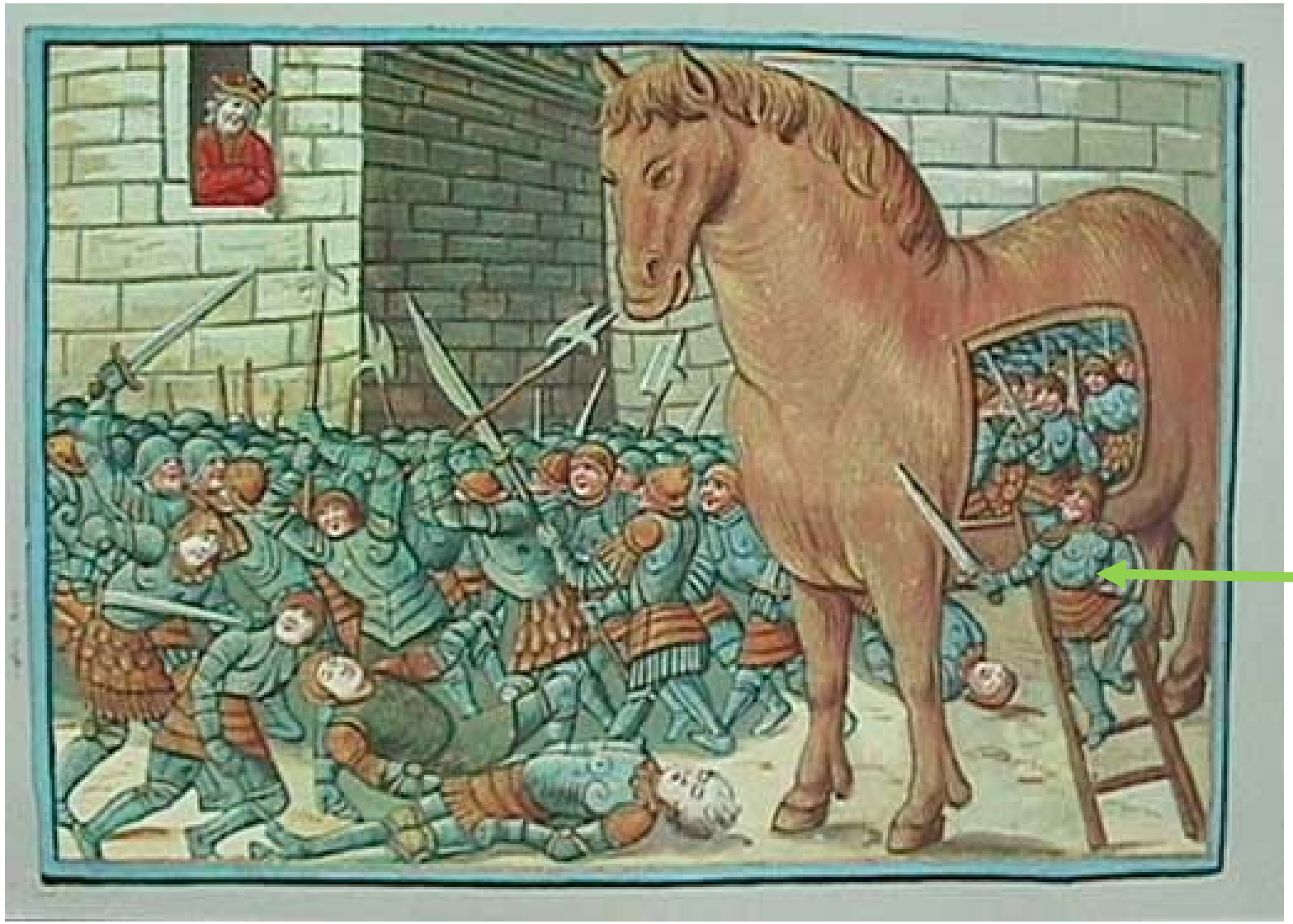


Some bacteria can escape host cells defense mechanisms



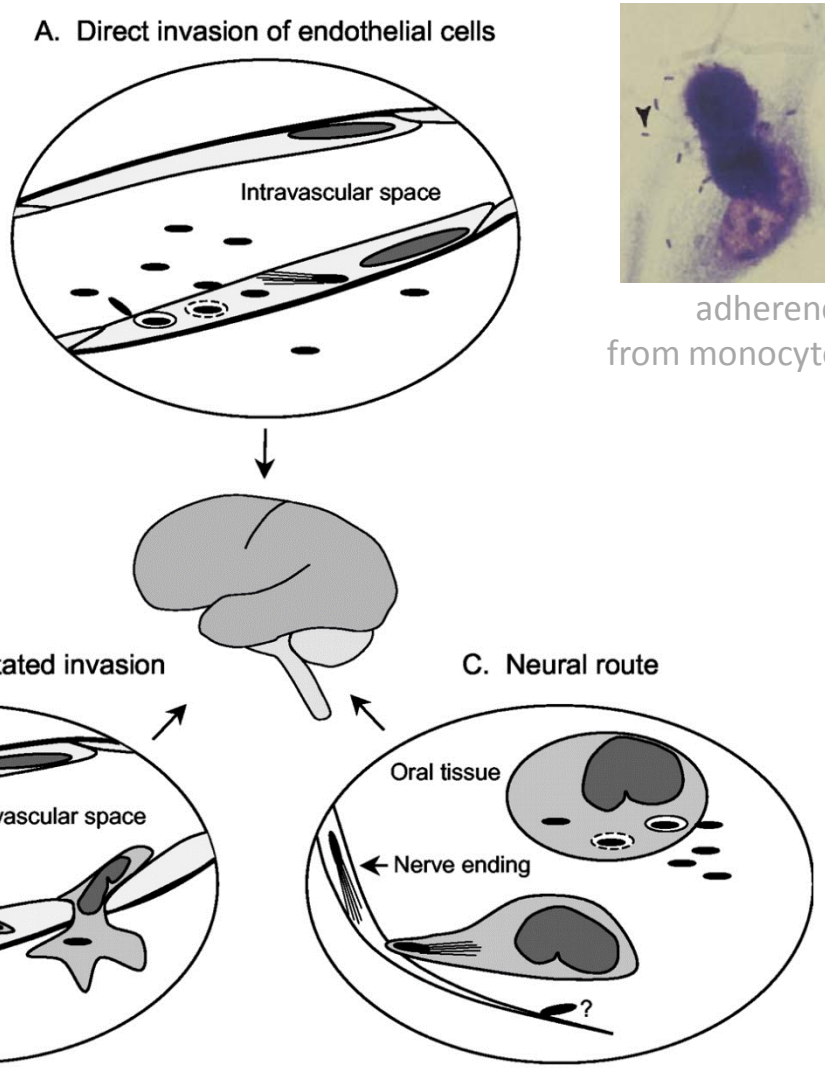
Carryn et al., Infect Dis Clin North Am. (2003) 17:615-34

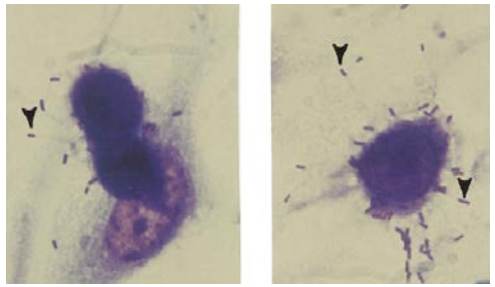
Benefits of intracellular life



Invasion of CNS by *Listeria monocytogenes*

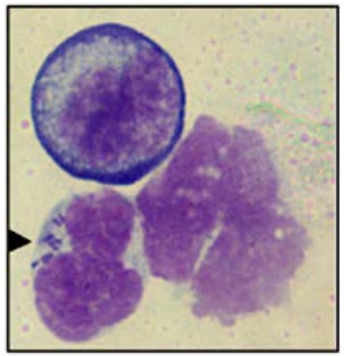
Listeria: from the gut to the CNS

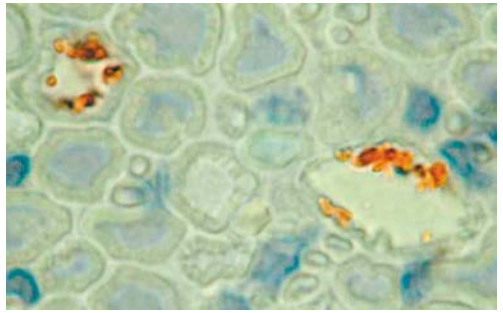




adherence and transfert
from monocytes to endothelial cells

bone-marrow monocyte

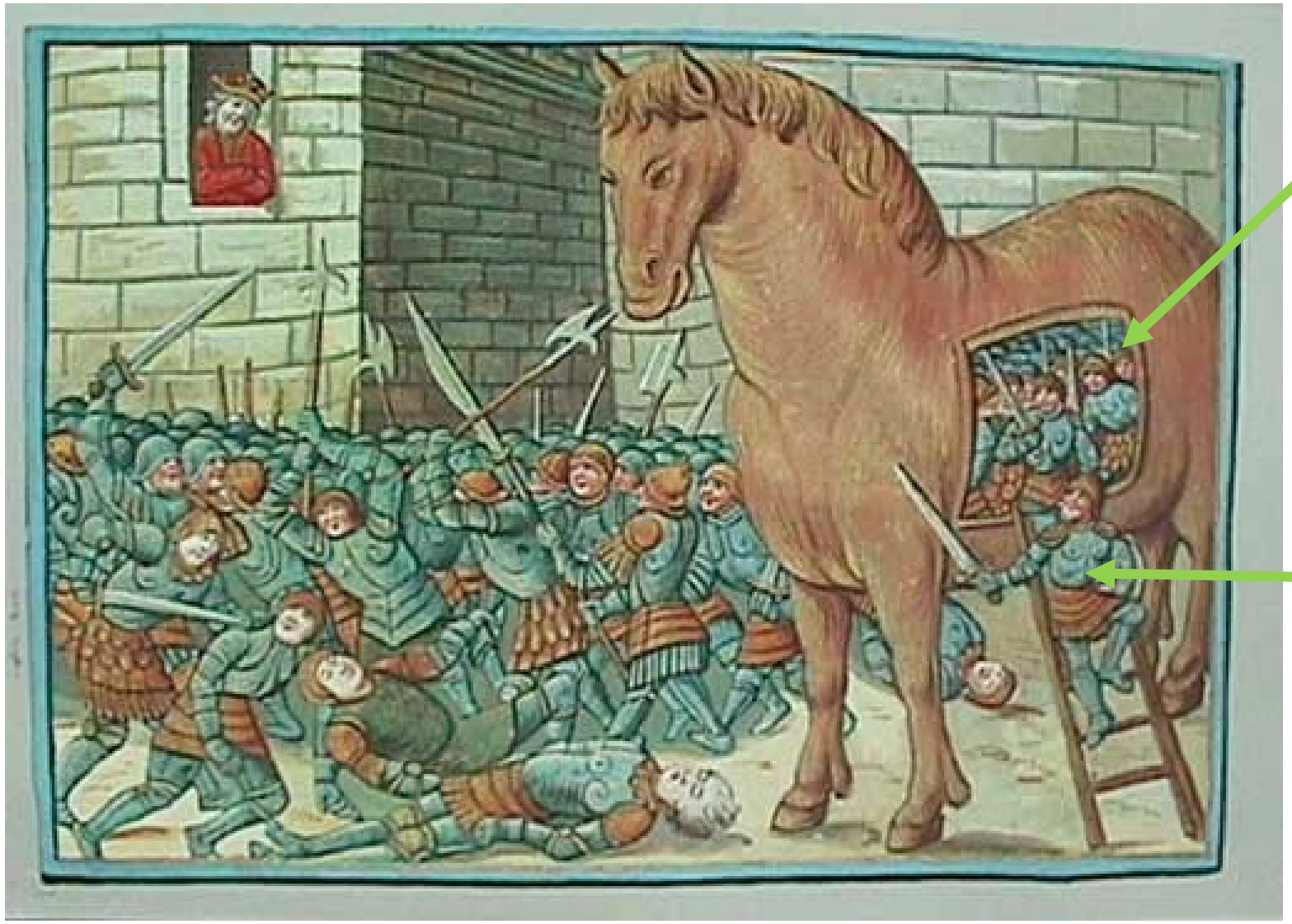




intra-axonal labeling
by anti-listeria antibodies

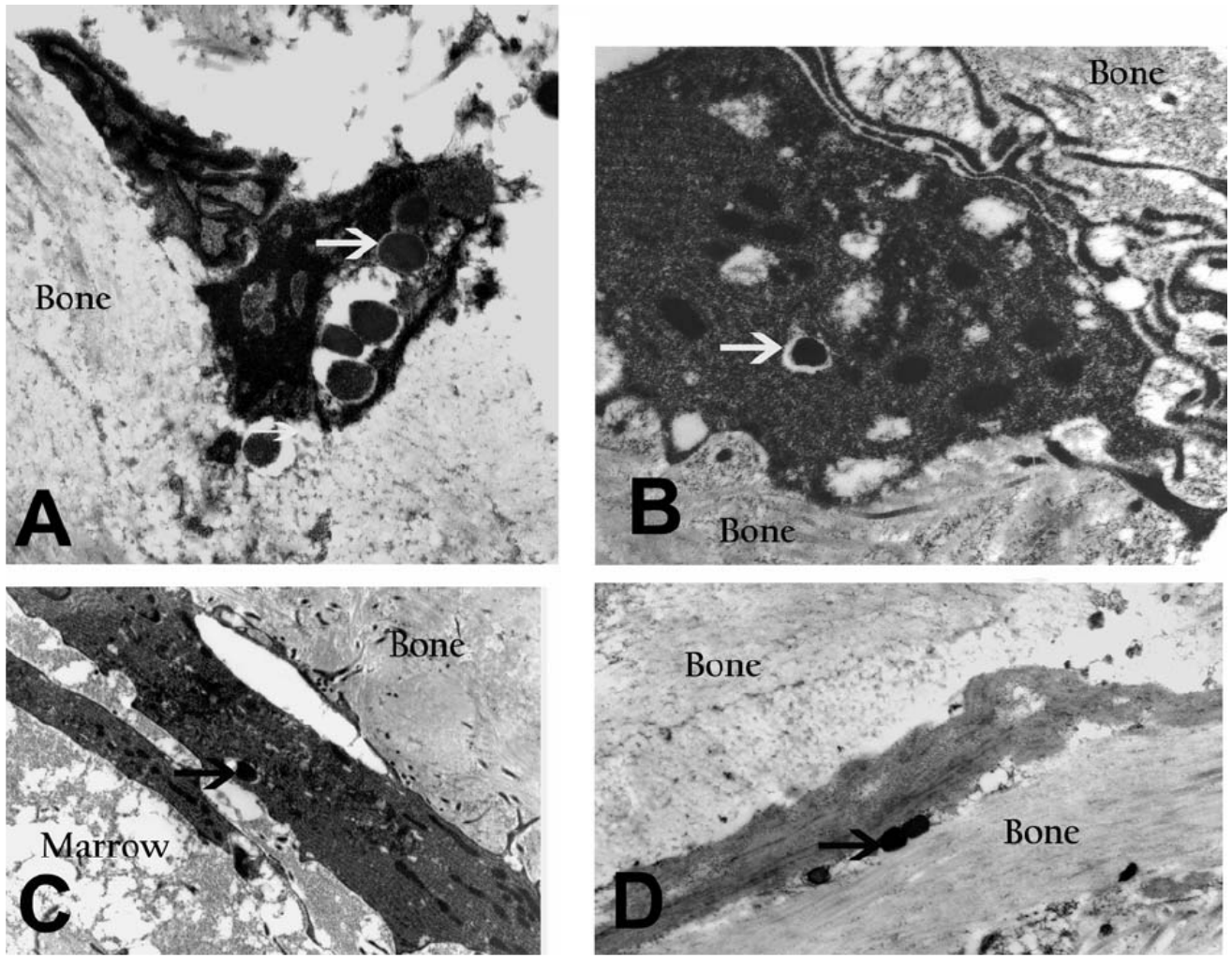
Antal et al., Brain Pathol. (2001) 11:432-8; Drevets & Bronze, FEMS Immunol Med Microbiol. (2008) 53:151-65
Drevets & Leenen, Microbes Infect. (2000) 2:1609-18; Drevets et al., Clin. Microb. Rev. (2004) 17:323-47

Benefits of intracellular life



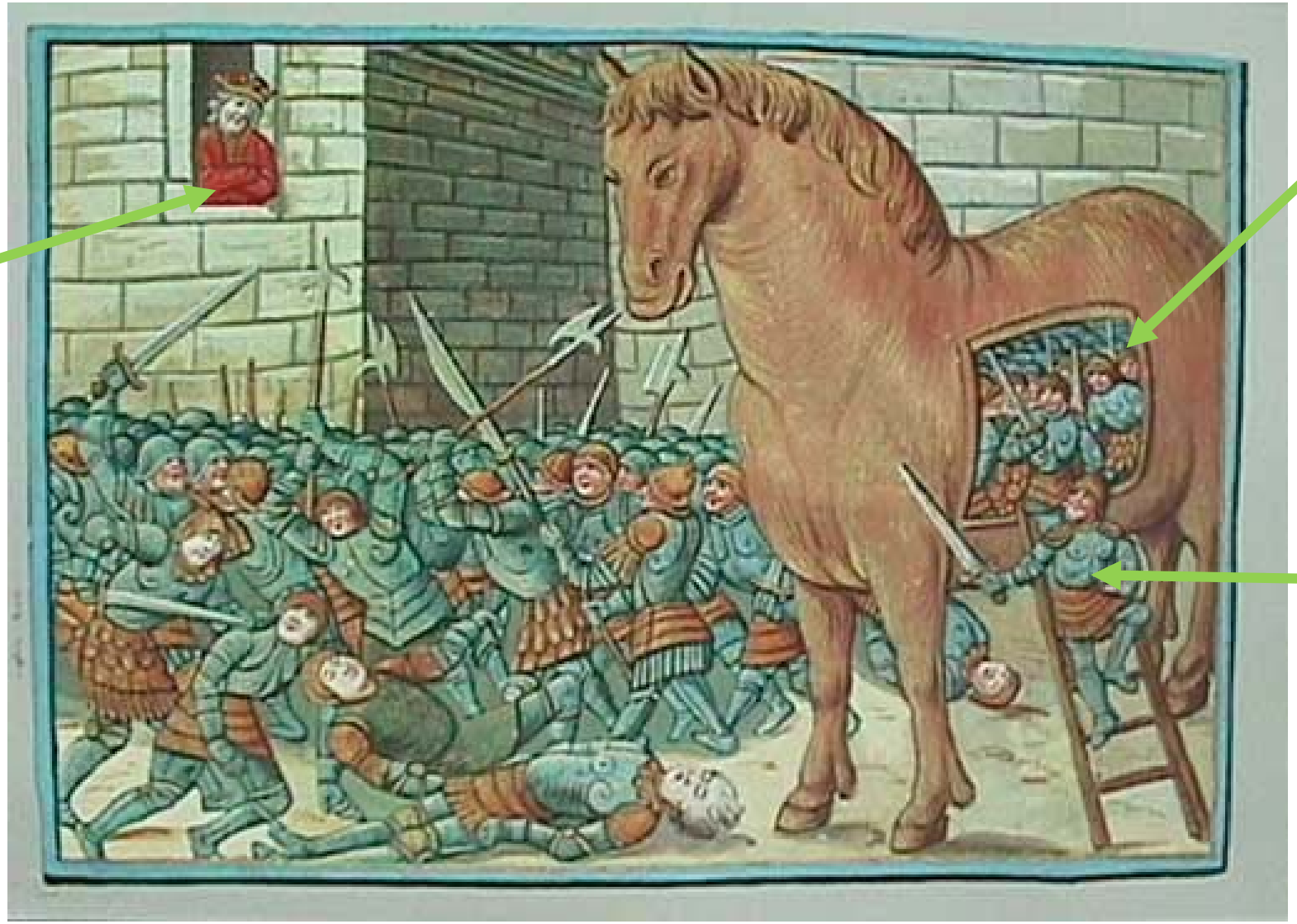
Persistent infection by *S. aureus*

Evidence of an intracellular reservoir in osteocytes (A,B), osteoblasts (C) and bone matrix of a patient with recurrent osteomyelitis



Bosse et al., J Bone Joint Surg Am. (2005) 87:1343-7

Benefits of intracellular life



Failures to eradicate intracellular bacteria with antibiotics

Reduced Ability of Penicillin to Eradicate Ingested Group A Streptococci from Epithelial Cells: Clinical and Pathogenetic Implications

Edward L. Kaplan,[1,a] Gursharan S. Chhatwal,[2] and Manfred Rohde[2]

[1]Department of Pediatrics, University of Minnesota Medical School, Minneapolis, Minnesota; and [2]Dep... Helmholtz Centre for Infection Research, Braunschweig, Germany

Clinical In...

Pathophysiology of chronic bacterial osteomyelitis. Why do antibiotics fail so often?

J Ciampolini and K G Harding

Postgrad Med J 2000 76: 479-483

BRIEF REPORTS • CID 2001:32 (1 June) • 1643

Intracellular Persistence of *Staphylococcus aureus* Small-Colony Variants within Keratinocytes: A Cause for Antibiotic Treatment Failure in a Patient with Darier's Disease

Christof von Eiff,[1] Karsten Becker,[1] Dieter Metze,[2] Gabriele Lubritz,[1] Johannes Hockmann,[2] Thomas Schwarz,[2] and Georg Peters[1]

[1]Institute of Medical Microbiology and [2]Department of Dermatology, ...ilhelms-Universität Münster, Münster, Germany

Infection. 1992 Mar-Apr;20(2):99-100.

Fatal Legionella pneumophila pneumonia: treatment failure despite early sequential oral-parenteral amoxicillin-clavulanic acid therapy.

Hohl P, Buser U, Frei R.

Dept. of Internal Medicine, University Hospital, Basel, Switzerland.

ORIGINAL ARTICLE

Electron microscopic evidence of persistent chlamydial infection following treatment

EY Bragina,† MA Gomberg,‡* GA Dmitriev†

†Department of Microbiology, Central Institute of Skin and Venereal Diseases, ‡Laboratory of Viral Urogenital Infections, Central Institute of Skin and Venereal Diseases, Korolenko Str., 3, Moscow, 107076, Russia.

JEADV (2001) 15, 405–409

Journal of Antimicrobial Chemotherapy (2004) 53, 167–173
DOI: 10.1093/jac/dkh076
Advance Access publication 16 January 2004

Antibiotic-induced persistence of cytotoxic *Staphylococcus aureus* in non-phagocytic cells

Oleg Krut, Herdis Sommer and Martin Krönke*

Pediatr Infect Dis J. 2006 Oct;25(10):880-3.

Persistence of erythromycin-resistant group a streptococci in cultured respiratory cells.

Spinaci C, Magi G, Varaldo PE, Facinelli B.

Institute of Microbiology and Biomedical Sciences, Marche Polytechnic University Medical School, Ancona, Italy.

OPEN ACCESS Freely available online

PLoS one

Penicillin Induced Persistence in *Chlamydia trachomatis*: High Quality Time Lapse Video Analysis of the Developmental Cycle

Rachel J. Skilton[¤], Lesley T. Cutcliffe[¤], David Barlow, Yibing Wang, Omar Salim, Paul R. Lambden, Ian N. Clarke*

Molecular Microbiology Group, University of Southampton Medical School, Southampton General Hospital, Southampton, United Kingdom

Intracellular models for antimicrobial R&D

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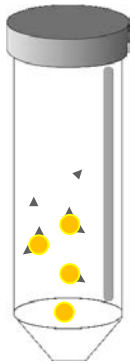
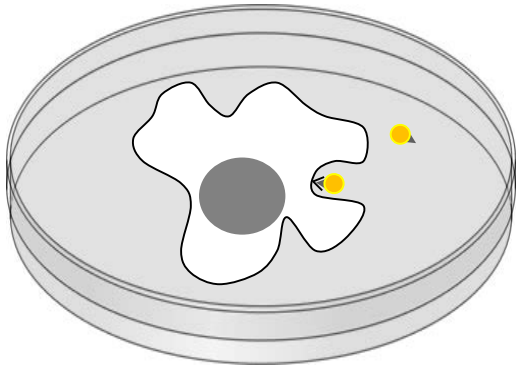
In vitro model of intracellular infection

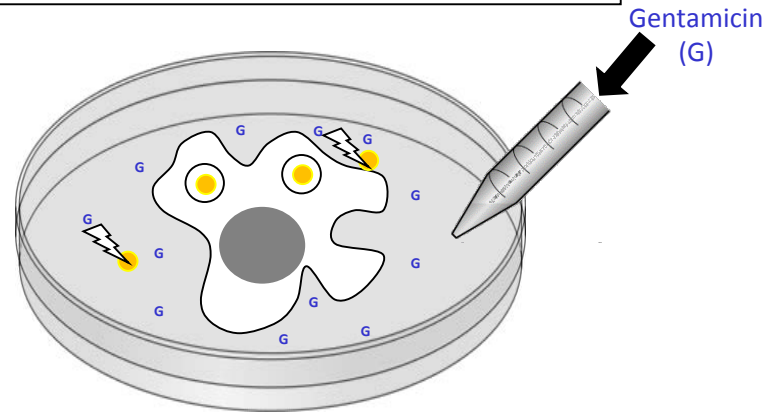
Opsonization
Incubation
with 10 % human serum
in culture medium



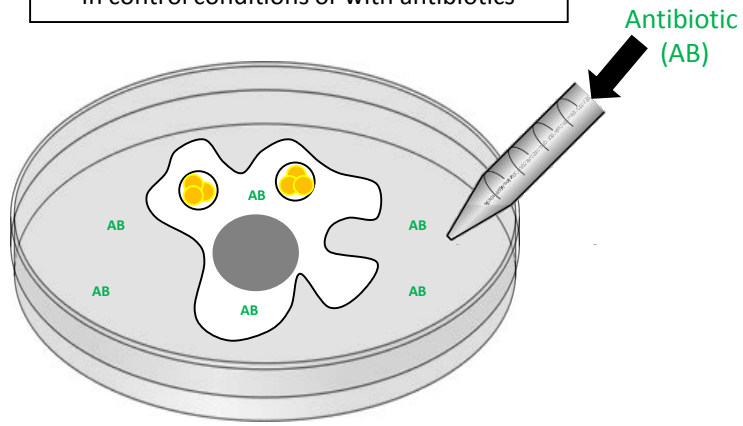
Phagocytosis
Incubation of bacteria with
cells at an appropriate MOI



Extracellular wash
Elimination of non phagocytosed bacteria
by incubation with 50-100 X MIC gentamicin;
elimination of gentamicin by washing

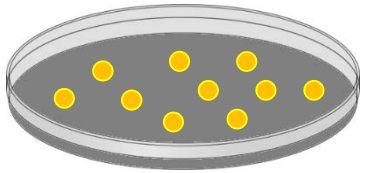


Intracellular infection
Incubation over time
in control conditions or with antibiotics

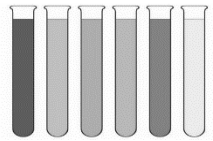


Determination of residual intracellular inoculum
Determination of cfu and protein content in
cell lysates

cfu



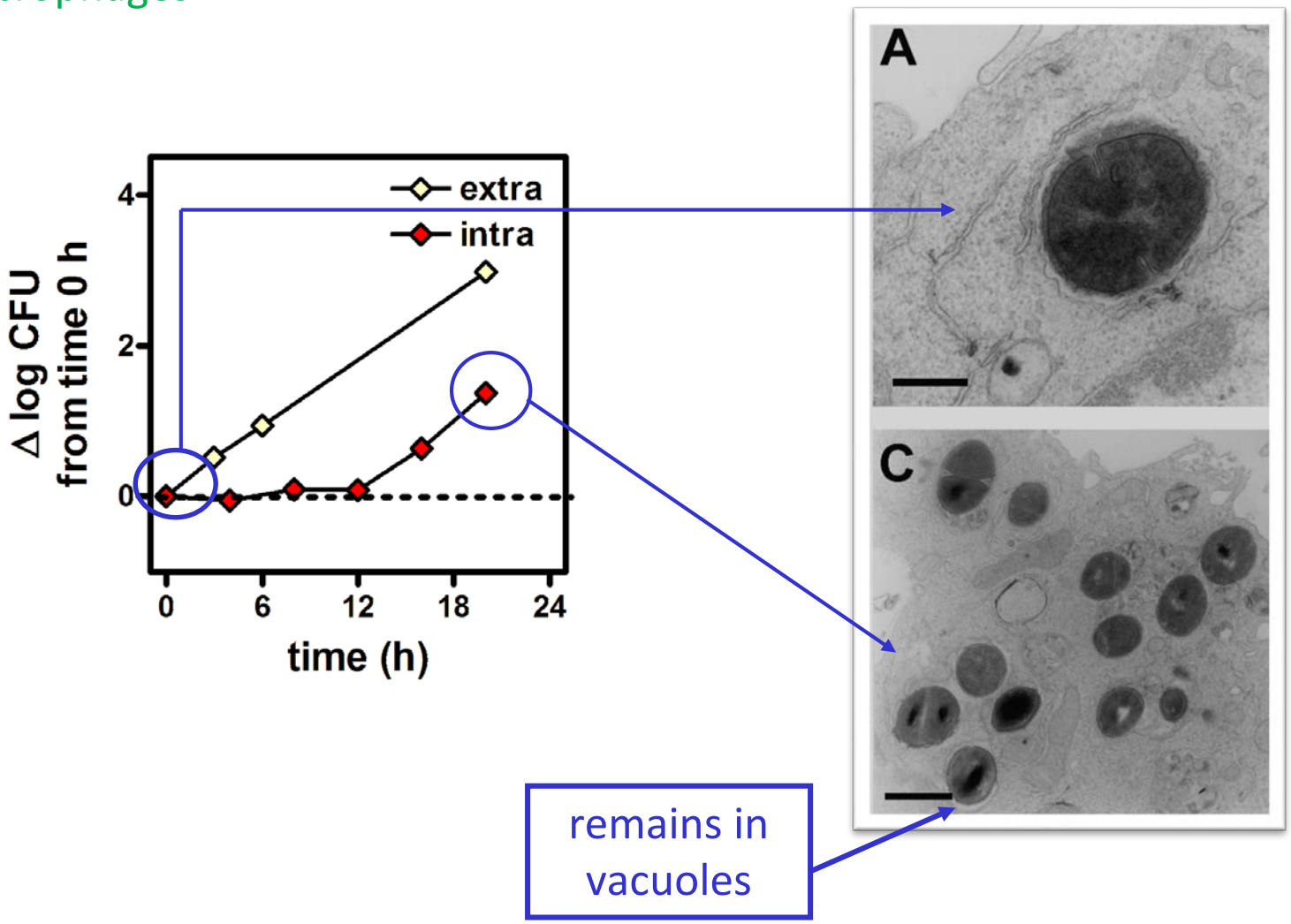
proteins



Buyck et al, 2016; ISBN 978-1-4939-2854-5

In vitro model of intracellular infection

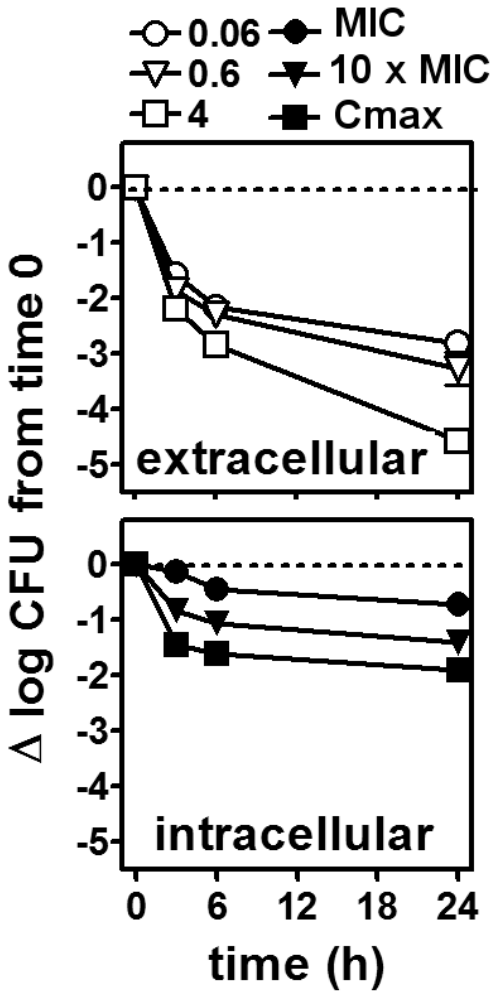
S. aureus in J774 macrophages

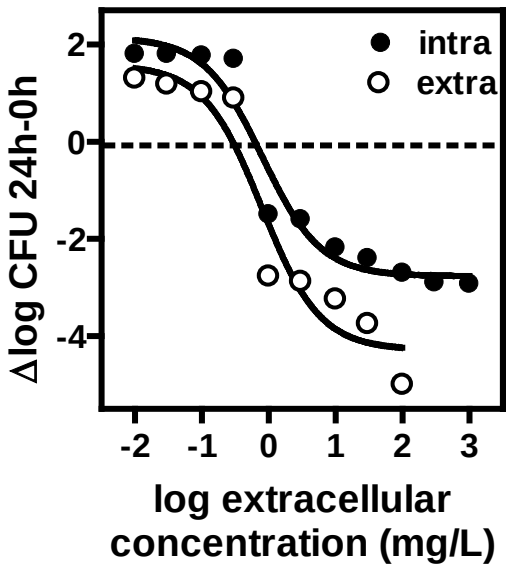


Seral et al, AAC (2003) 47:2283-92

Setting-up appropriate models for the study of antibiotic activity against intracellular bacteria

moxifloxacin & *S. aureus*

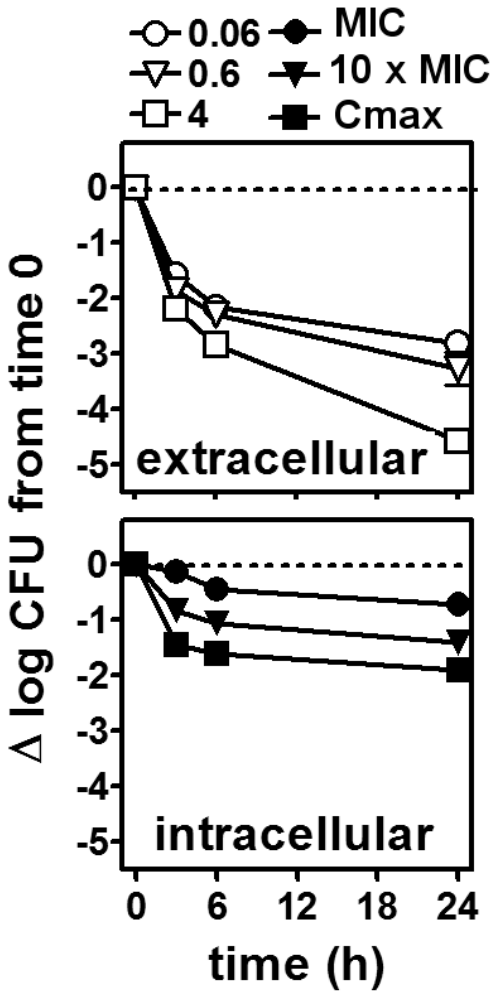


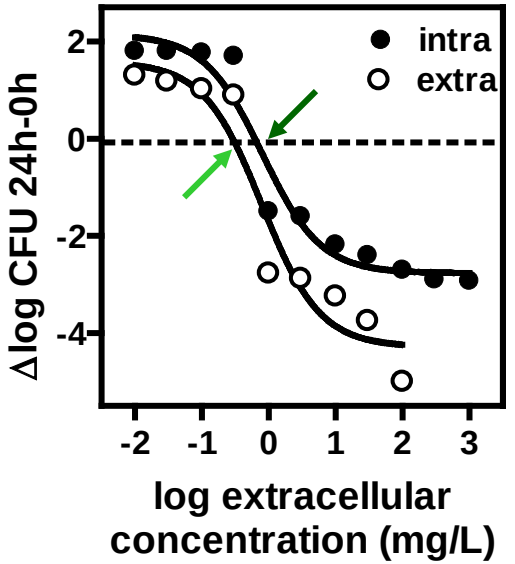


Barcia-Macay et al, AAC (2006) 50:841-51

Setting-up appropriate models for the study of antibiotic activity against intracellular bacteria

moxifloxacin & *S. aureus*





model	C_{stat} (x MIC)
extra	0.27
intra	0.63

relative potency

Barcia-Macay et al, AAC (2006) 50:841-51

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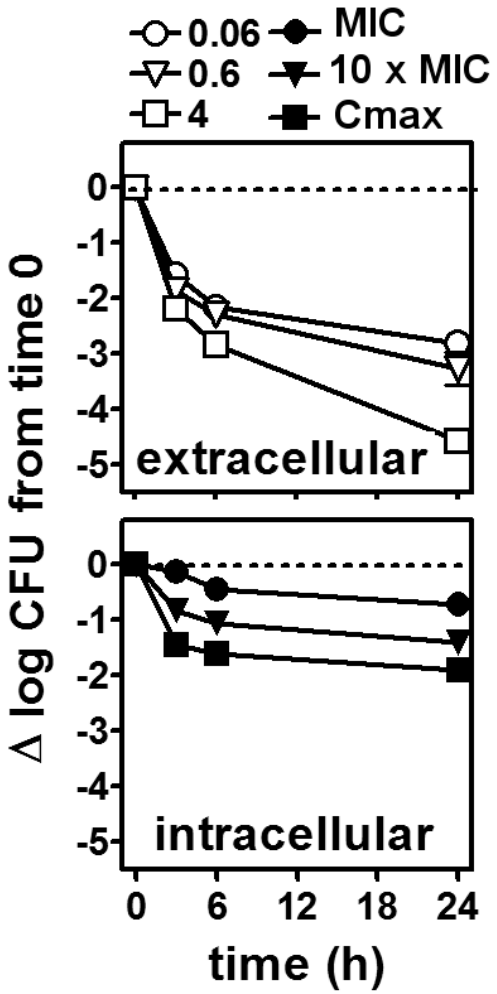
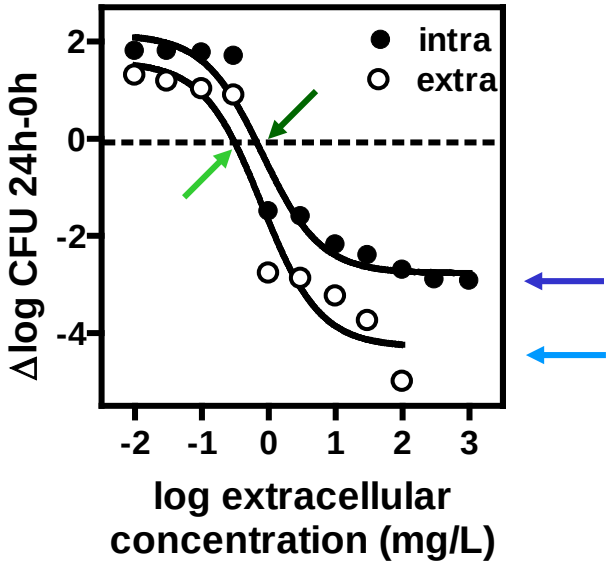




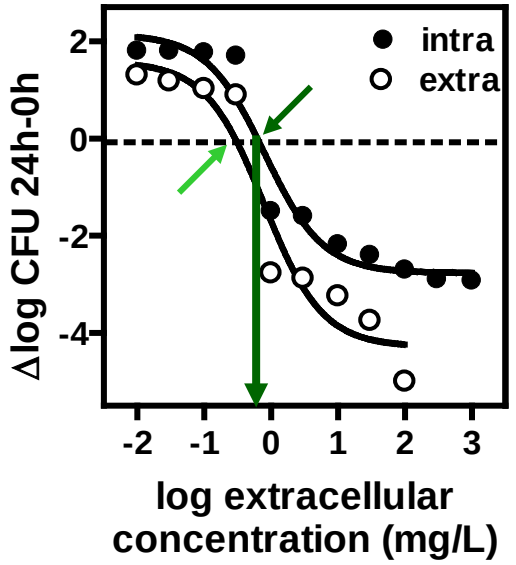
model	C_{stat} (x MIC)	E_{max}
extra	0.27	-3.86 (5.22 to 2.51)
intra	0.63	-2.77 (3.31 to 2.22)

relative potency

maximal efficacy

Barcia-Macay et al, AAC (2006) 50:841-51

What do these parameters tell you ?



relative potency

- Estimation of the concentration needed to reach a specified effect
- Measure of the « intracellular MIC »

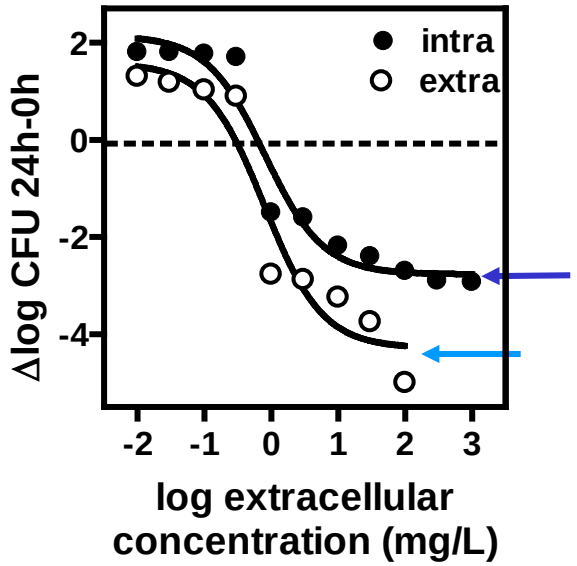
⇨ « PK-related » parameter:
- accumulation in the infected compartment
- intracellular bioavailability

⇨ influence of local environment on intrinsic activity
- pH
- oxidant species

In most cases
Cs intra ≥ Cs extra

What do these parameters tell you ?

maximal efficacy

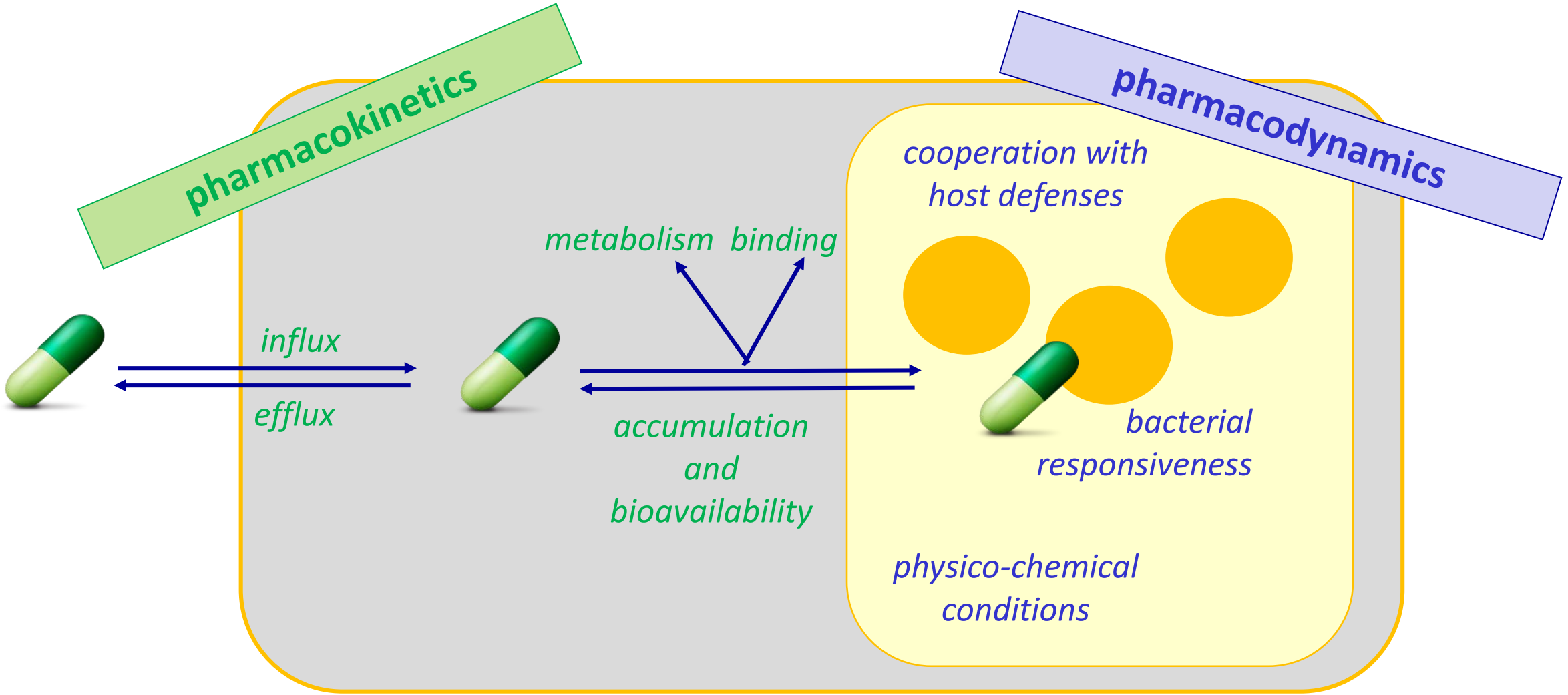


- Estimation of the maximal reduction in inoculum for an infinitely large concentration
- Measure of the killing capacity

⇨ « PD-related » parameter
- mode of action of the drug
- bacterial responsiveness
- cooperation with host defenses

In most cases
Emax intra <<< Emax extra

PK/PD parameters and intracellular activity



Carryn et al, Infect Dis Clin North Am (2003) 17:615-34

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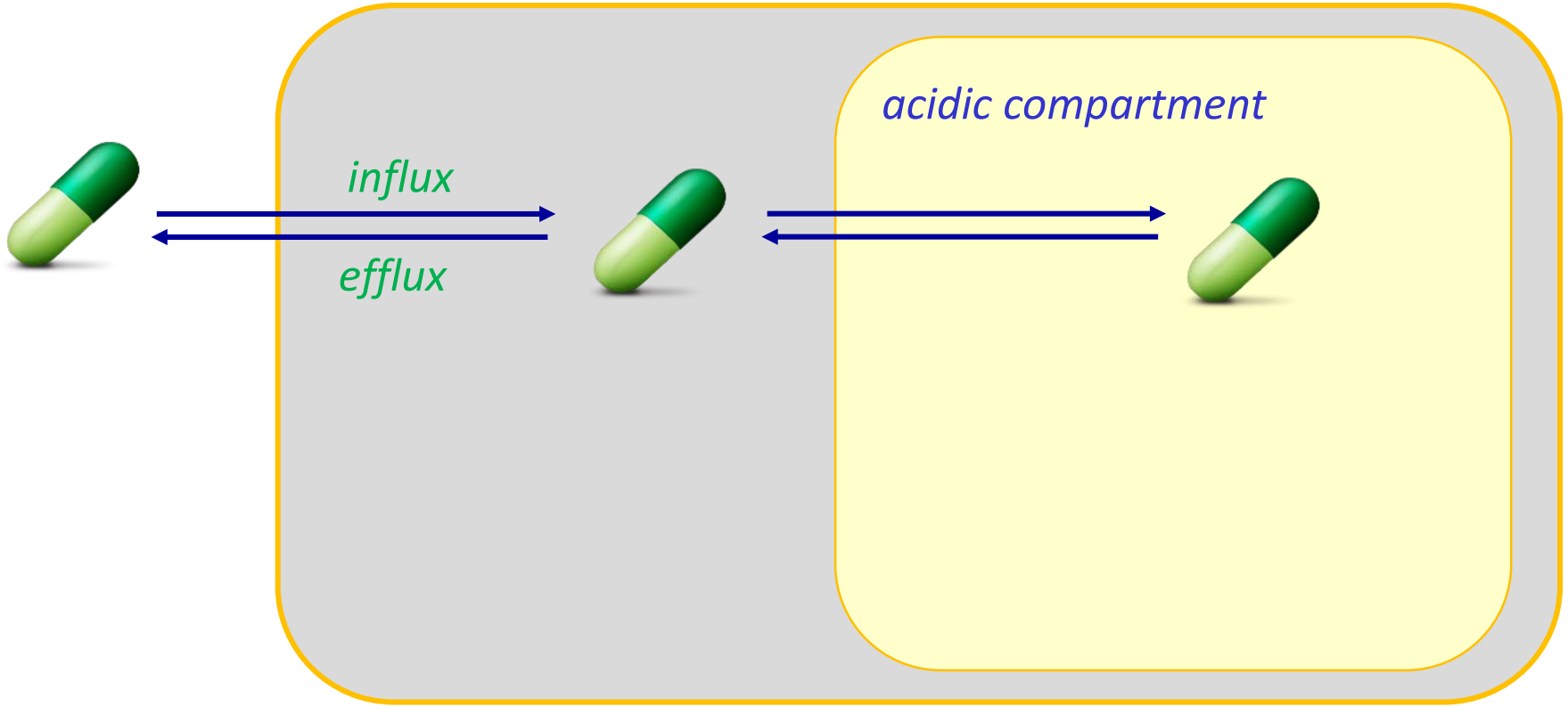
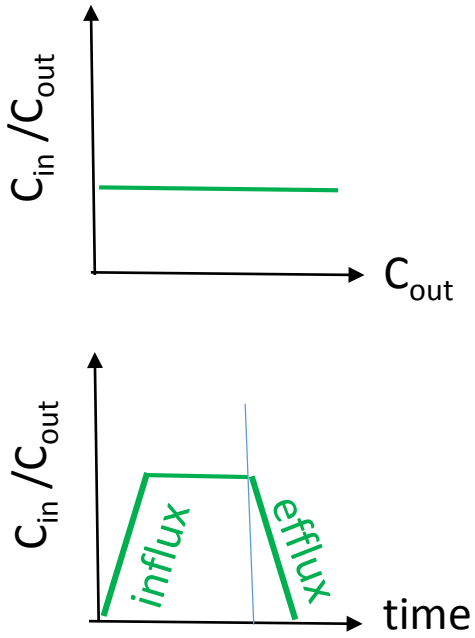
Mechanisms by which antibiotics can enter/distribute inside/leave eukaryotic cells

1. Simple diffusion



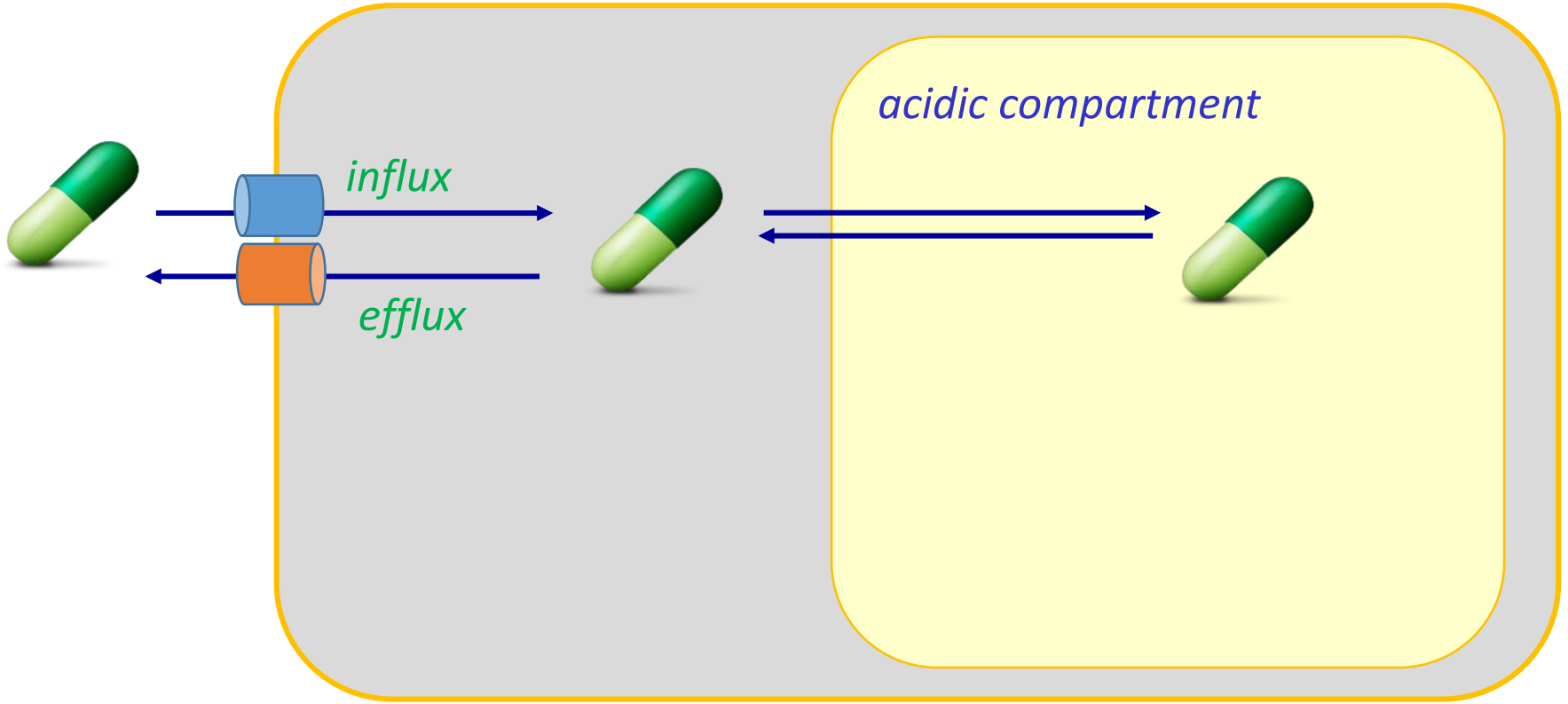


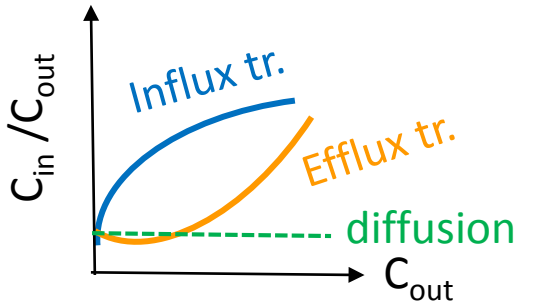
- C_{in} = C_{out} except if “binding site”
- Linearly related to C_{out}
- Rapid process; rate of influx = rate of efflux
- Probably access to different compartments

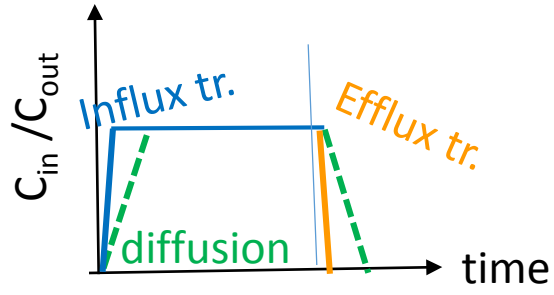
(β-lactams)

Mechanisms by which antibiotics can enter/distribute inside/leave eukaryotic cells

2. Transporters







Influx:

- Accumulation lower for higher C_{out} (saturation)
- More rapid than diffusion

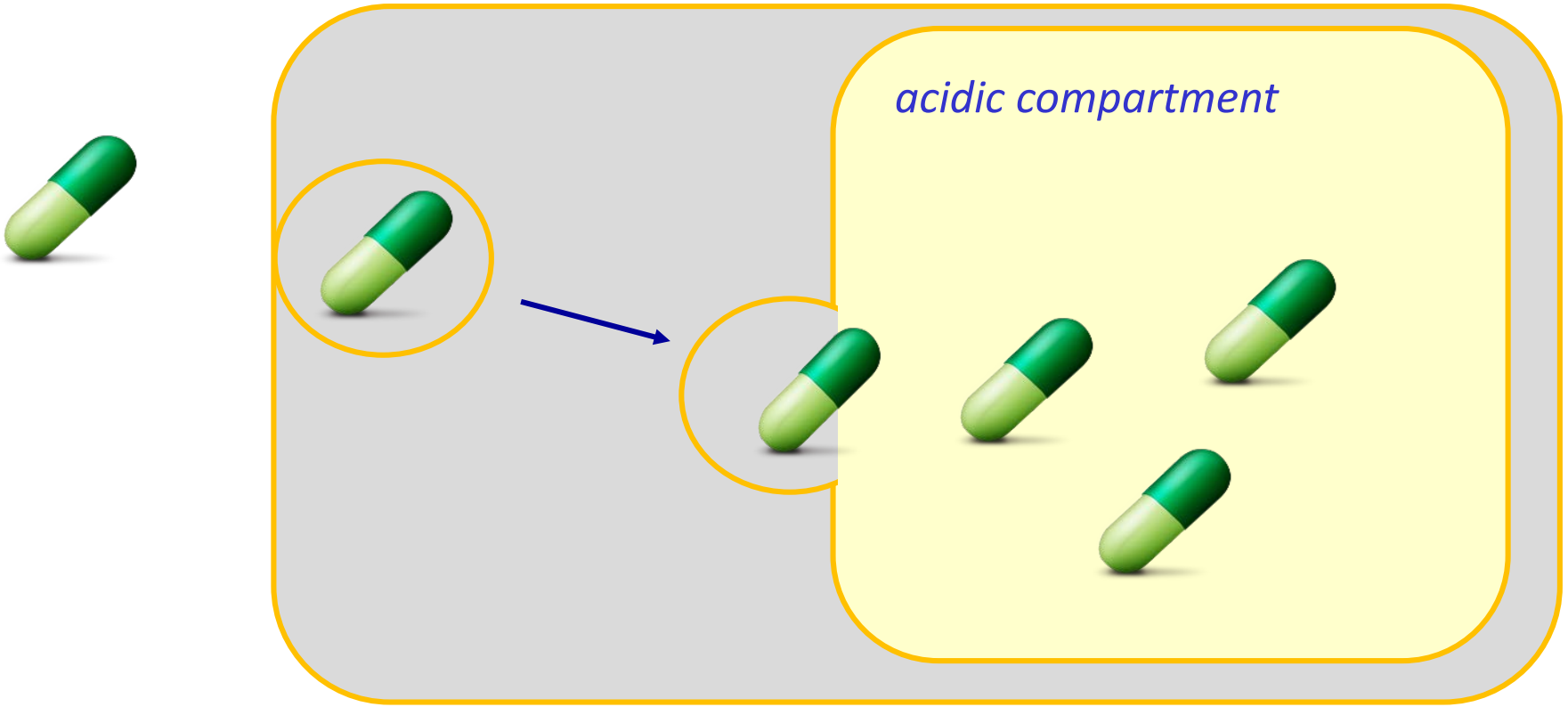
Efflux:

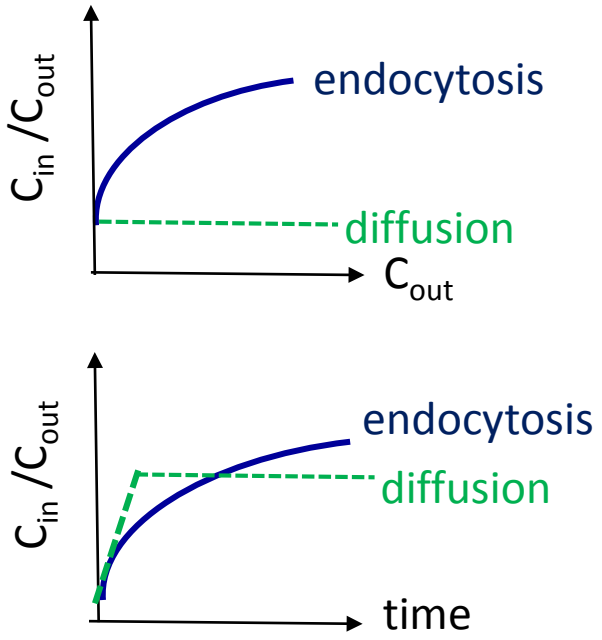
- Accumulation higher for higher C_{out} (saturation)
- More rapid than diffusion

(fluoroquinolones)

Mechanisms by which antibiotics can enter/distribute inside/leave eukaryotic cells

3. Endocytosis



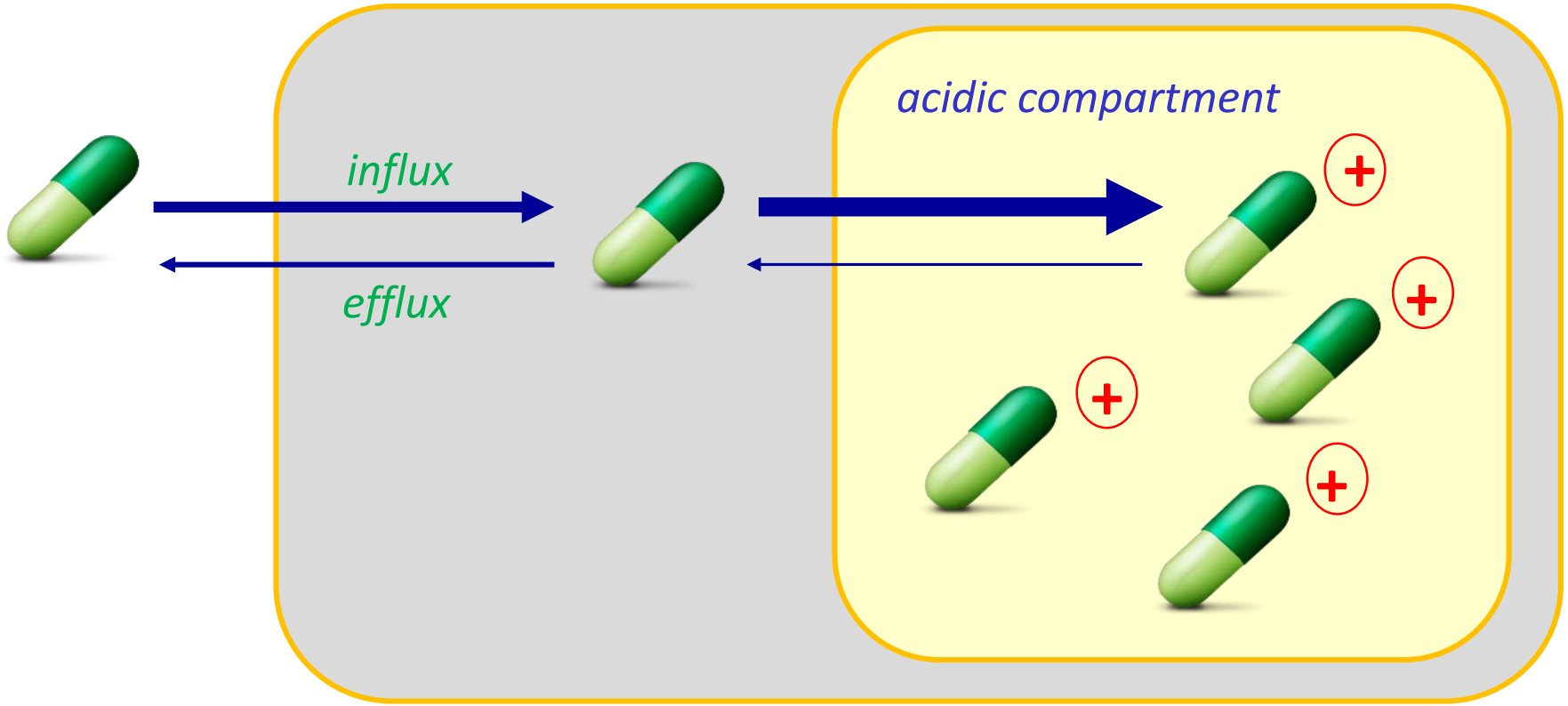


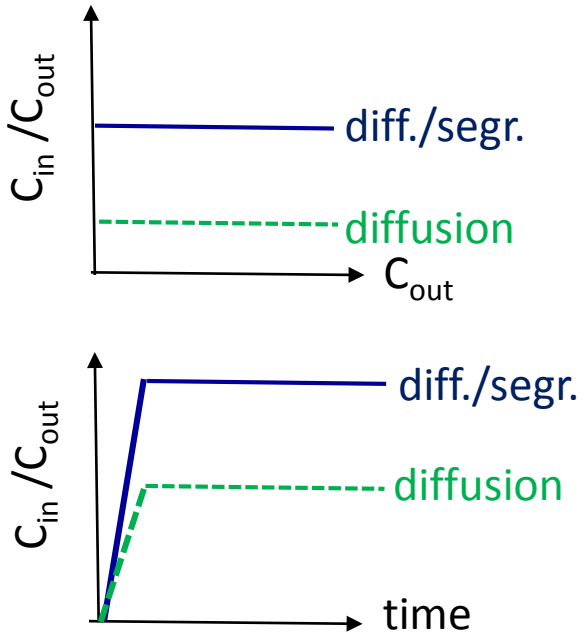
- Slow and saturable if adsorption at cell surface (~ transporter)
- Antibiotic confined in acidic vacuoles
- No or slow release

(aminoglycosides)

Mechanisms by which antibiotics can enter/distribute inside/leave eukaryotic cells

4. Diffusion/segregation



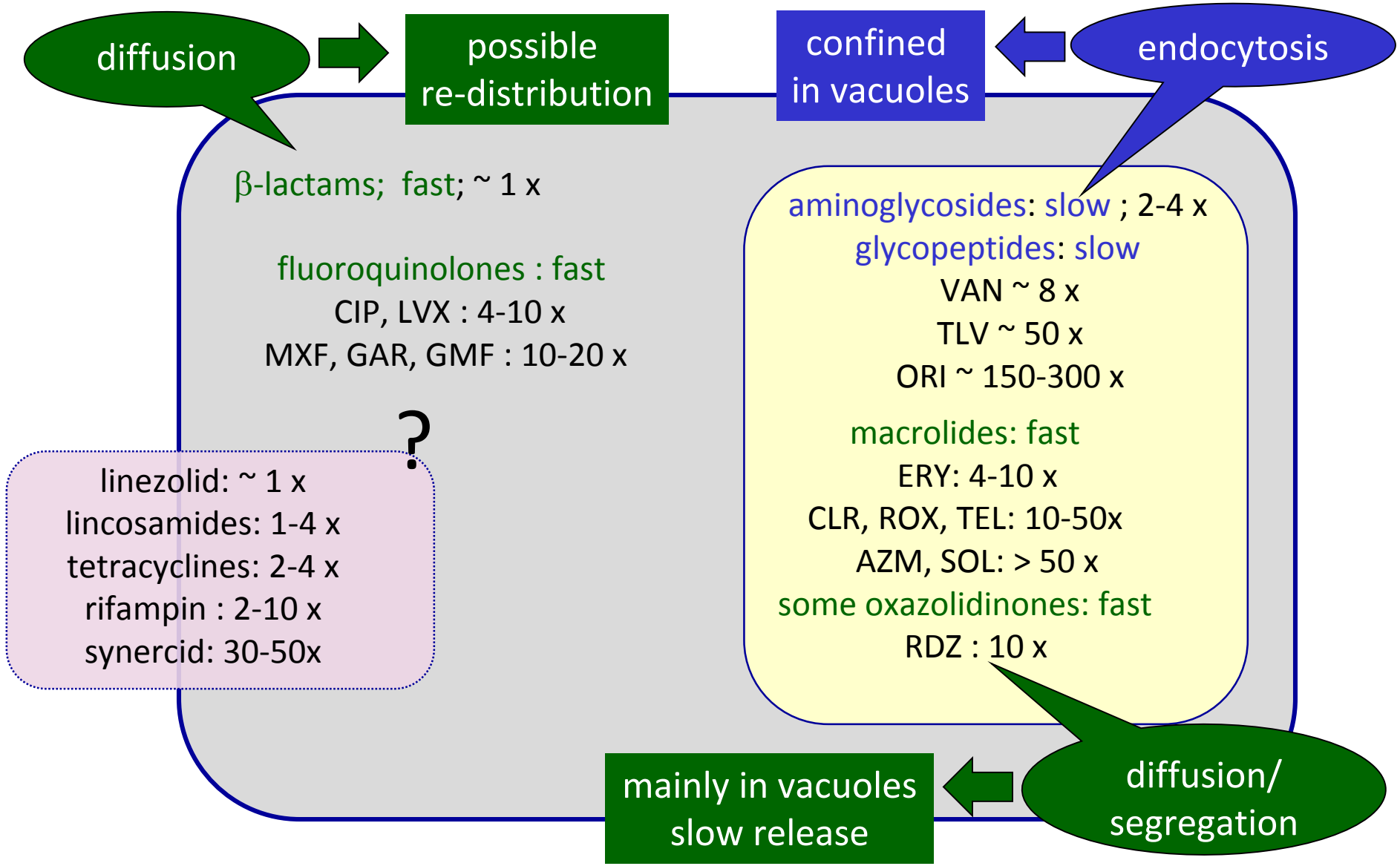


Typical for weak basic compounds

- Rapid and high accumulation
- Preferential accumulation (segregation) in acidic compartments
- Slow release

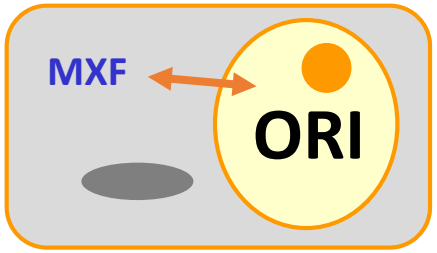
(macrolides)

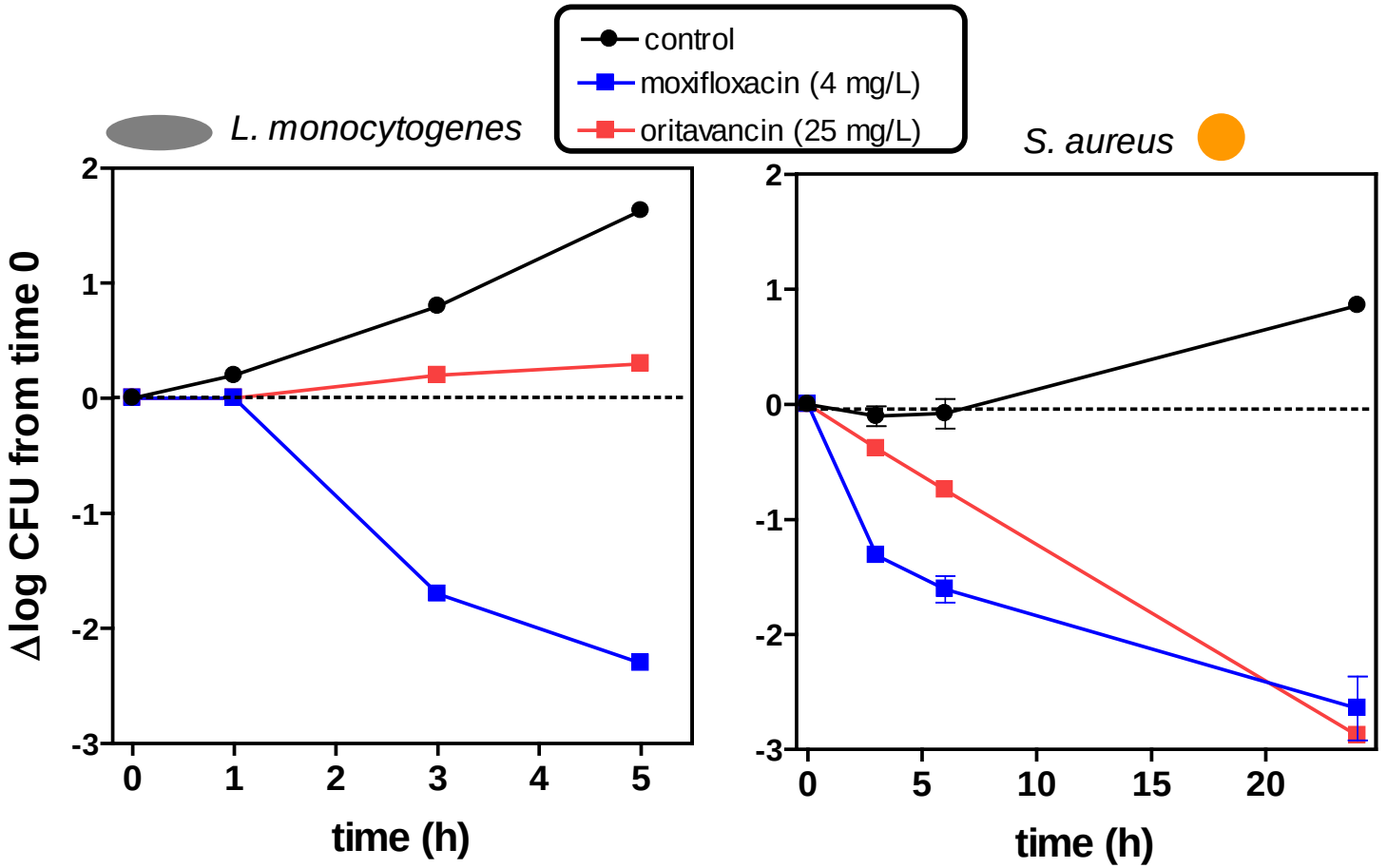
A summary of current data



Importance of subcellular distribution

Moxifloxacin & oritavancin against *L. monocytogenes* vs. *S. aureus*

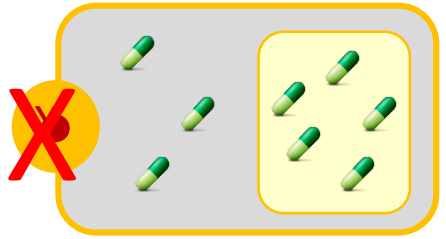




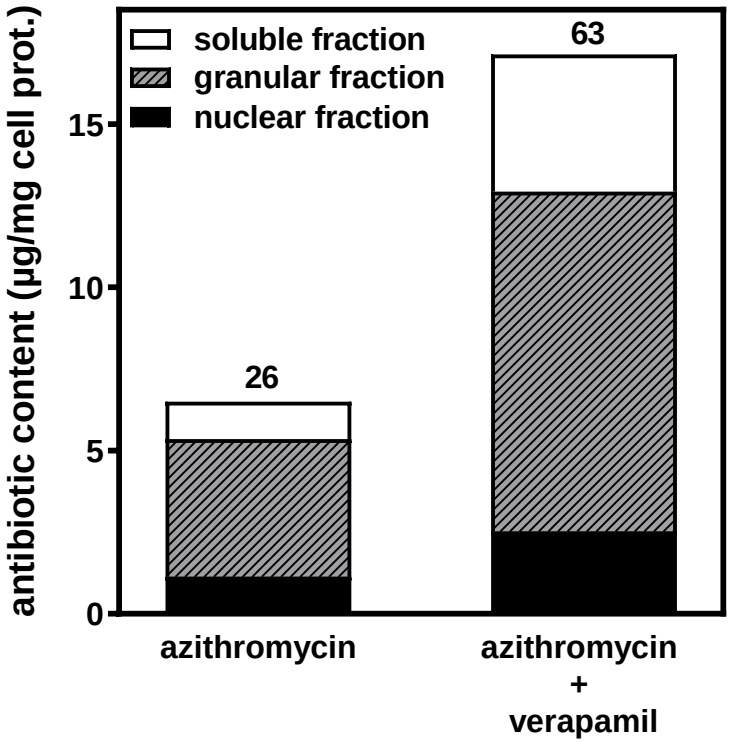
AB needs to have access to the infected compartment

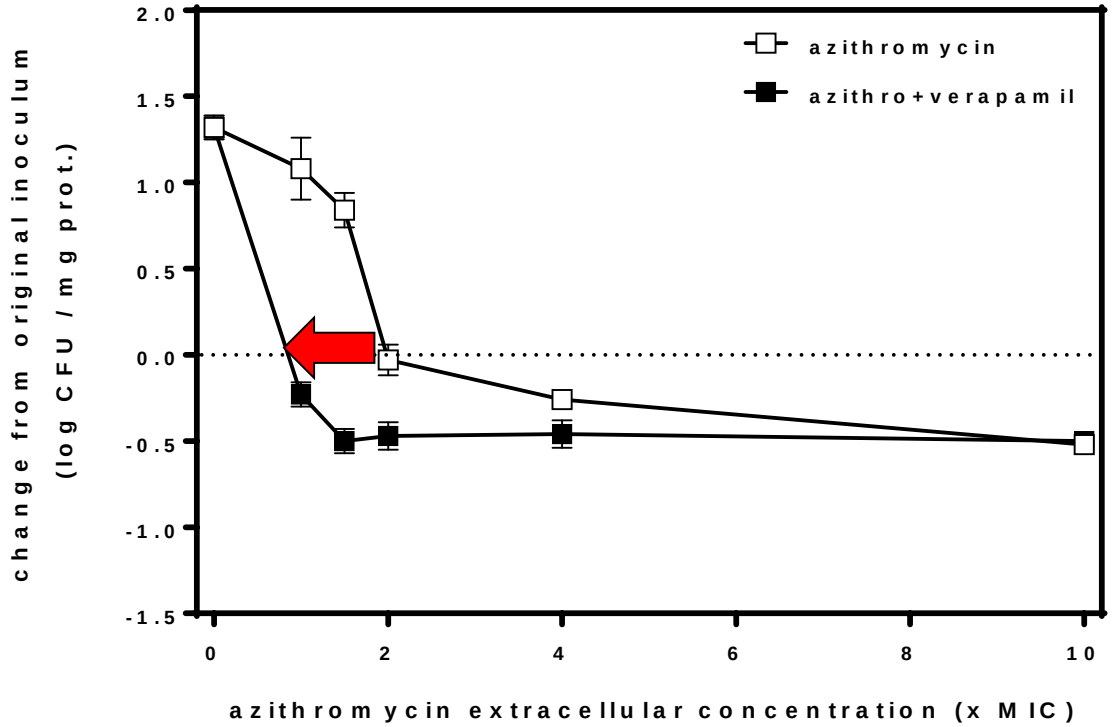
adapted from Carryn et al, AAC (2002) 46:2095-2103
Van Bambeke et al, AAC (2004) 48:2853-60
Barcia-Macay et al, AAC (2006) 50:841-51

Increasing accumulation by inhibiting efflux



Verapamil is an inhibitor of P-glycoprotein
→ increase in azithromycin accumulation (cytosol/organelles)
→ Increase in relative potency against intracellular *S. aureus*

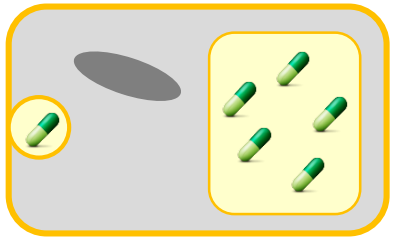




Inhibition of efflux increases relative potency

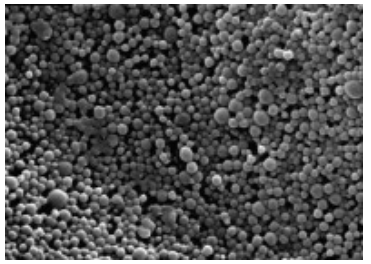
Seral et al. JAC (2003) 51:1167-73

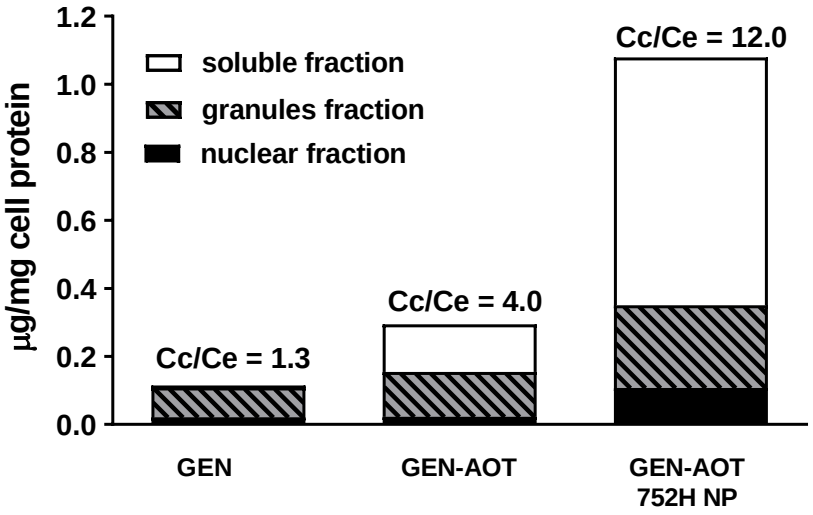
Modulation of distribution using adequate vectors

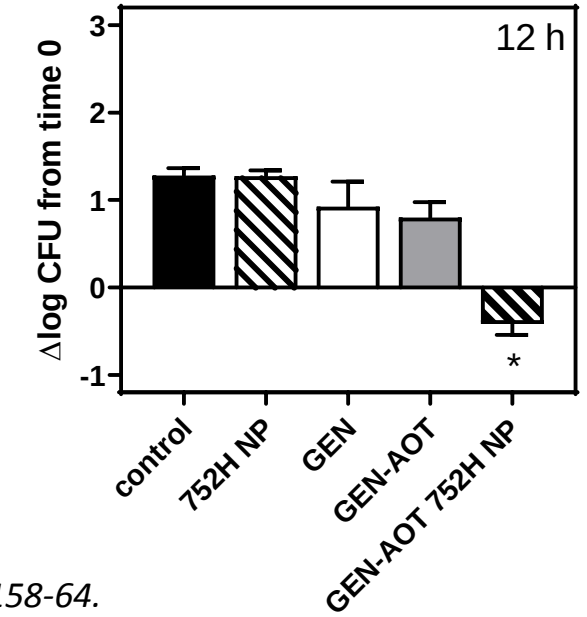


Aminoglycosides are not diffusible and accumulate in lysosomes by endocytosis
→ No activity against a cytosolic bacterium
→ Nanoparticle formulation for cytosolic release

gentamicin (GEN) + surfactant (AOT [bis(2-ethylhexyl) sulfosuccinate sodium salt])
+ poly(D,L-lactide-co-glycolide) (PLGA)





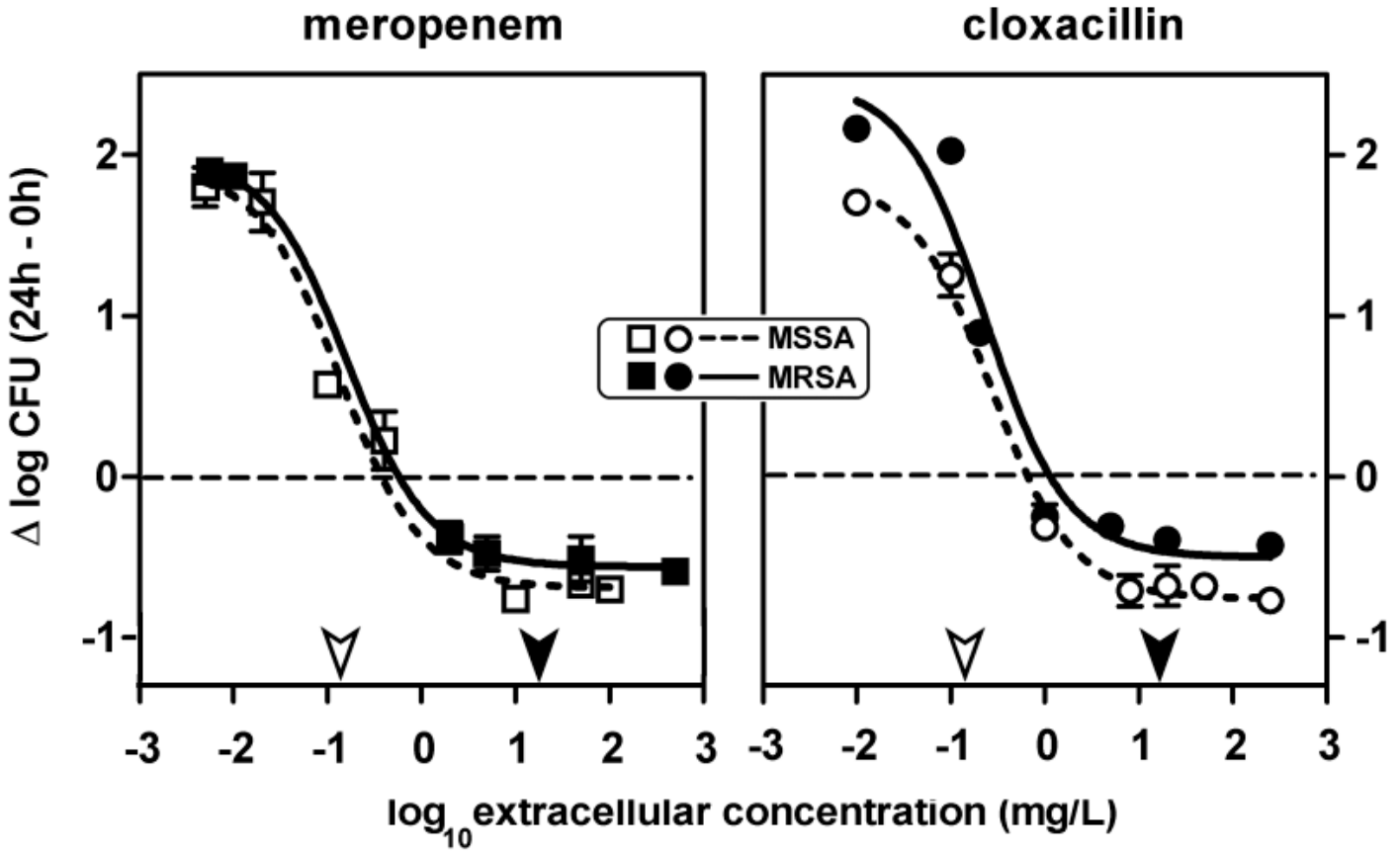


Increase in cytosolic concentration and in activity against a cytosolic bacterium

Imbuluzqueta et al. Acta Biomater. (2011) 7:1599-608; JAC (2012) 67:2158-64.

Impact of intracellular pH on intracellular potency (MIC)

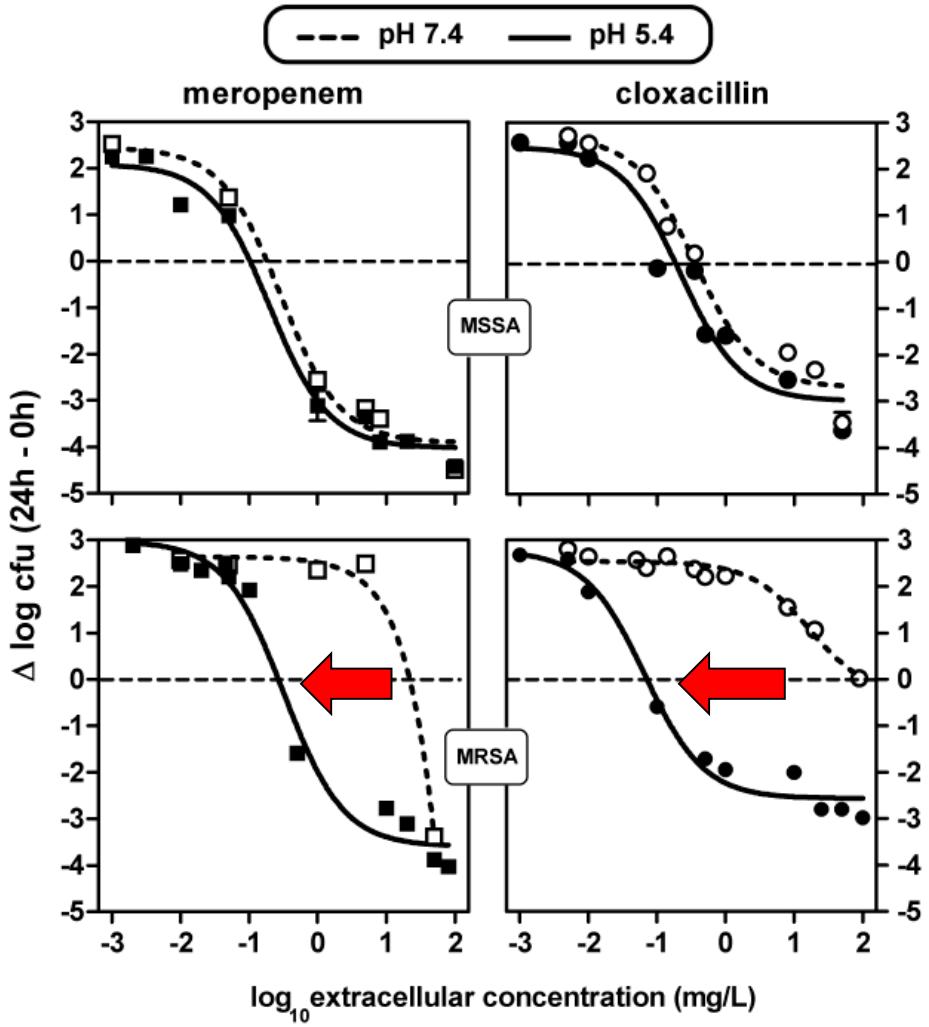
MRSA are as susceptible as MSSA to β-lactams when intracellular !



Lemaire et al., AAC (2007) 51:1627-32

Impact of intracellular pH on intracellular potency (MIC)

In broth, at acidic pH, MRSA are as susceptible as MSSA to β-lactams !



Lemaire et al., AAC (2007) 51:1627-32

Impact of intracellular pH on intracellular potency (MIC)

At acidic pH, the conformation of PBP2a is modified, allowing for the access of β-lactams !

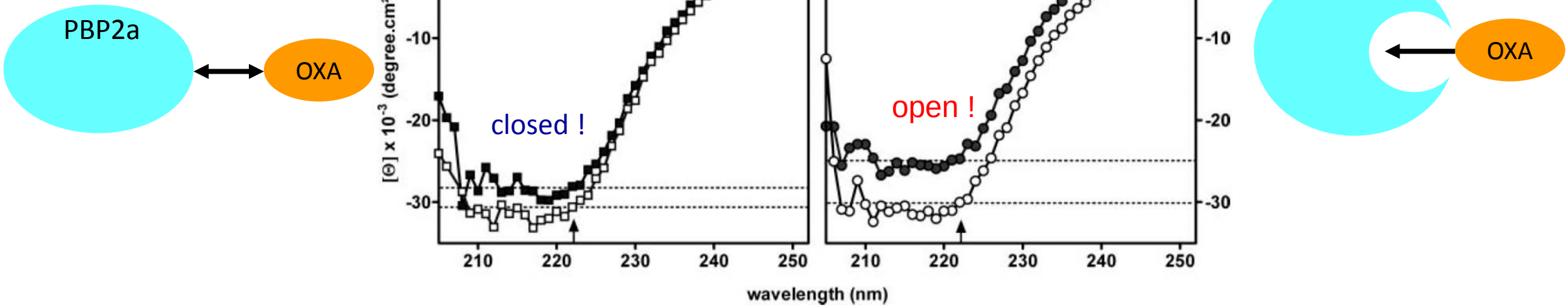


FIGURE 4. **Circular dichroic spectra of PBP 2a at pH 7. 0 (*left panel*) and pH 5.5 (*right panel*) in the absence (*open symbols*) and in the presence (*closed symbols*) of oxacillin (30 μM) for 30 min at 25 °C.** The *thin dotted lines* in each graph represent minima of PBP 2a molar ellipticity at 222 nm (*vertical arrow* on the *abscissa*) for each condition. The spectrum of oxacillin has been subtracted from all data points.

Lemaire et al., J Biol Chem (2008) 283:12769-76

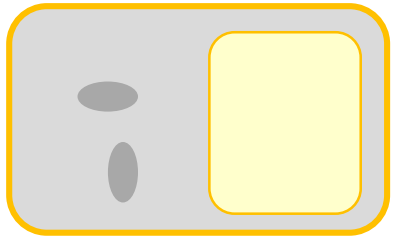
Intracellular models for antimicrobial R&D

- Role of intracellular survival in chronic infections and its contribution to poor response to antibiotics
- In vitro models to study intracellular activity of antibiotics
- Cellular pharmacokinetic (PK) parameters predictive of intracellular potency for antibiotics
- Cellular pharmacodynamic (PD) parameters predictive of intracellular efficacy for antibiotics

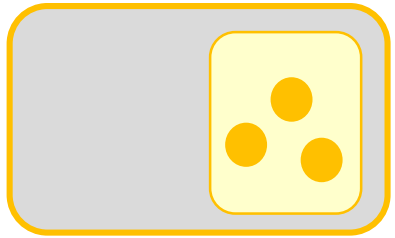
Bacterial responsiveness and PD

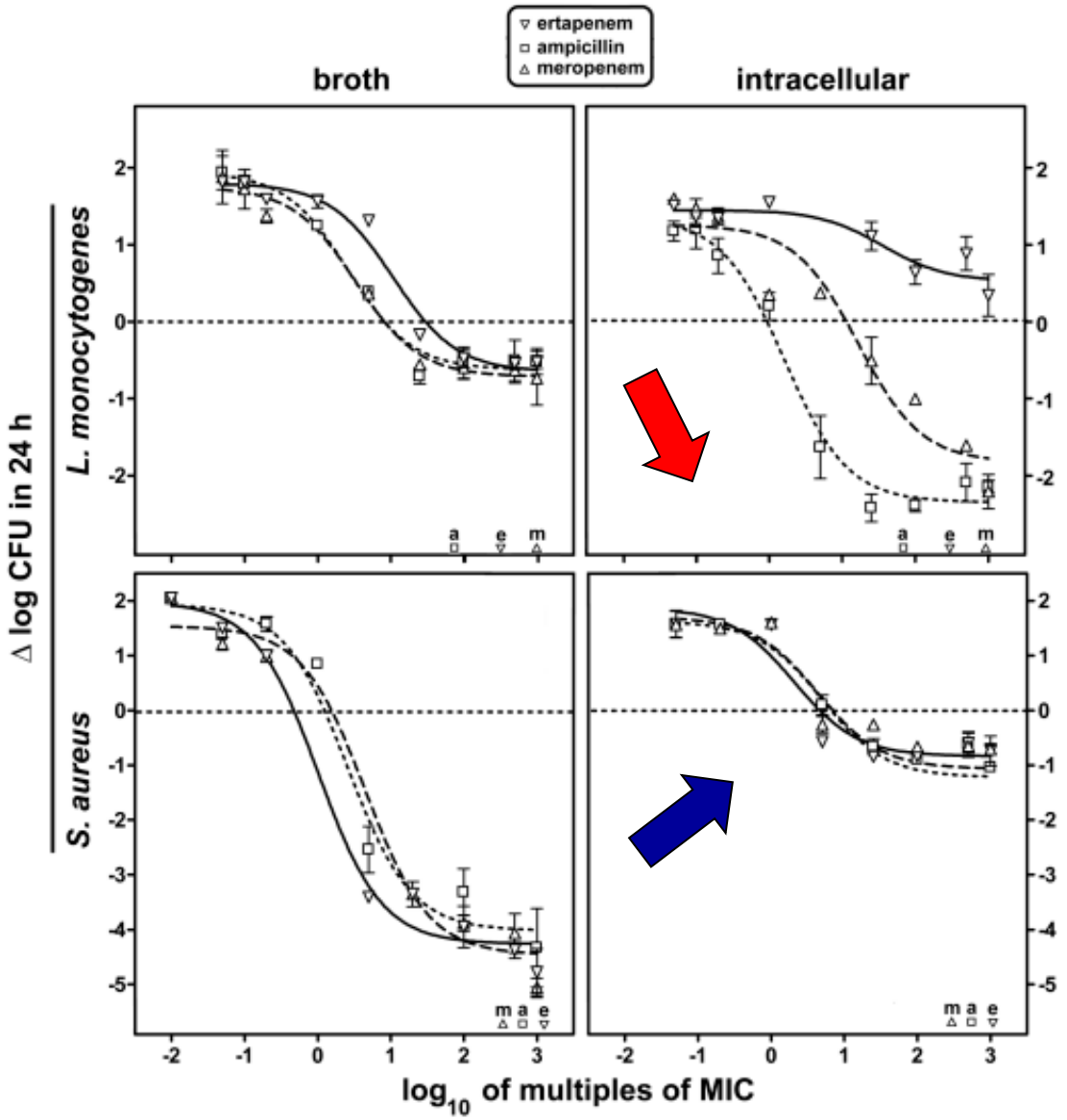
Differences among species

L. monocytogenes



S. aureus



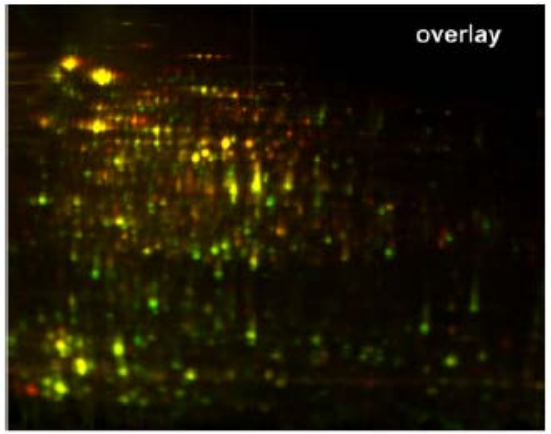


Why are β-lactams more active against intracellular *Listeria* ?

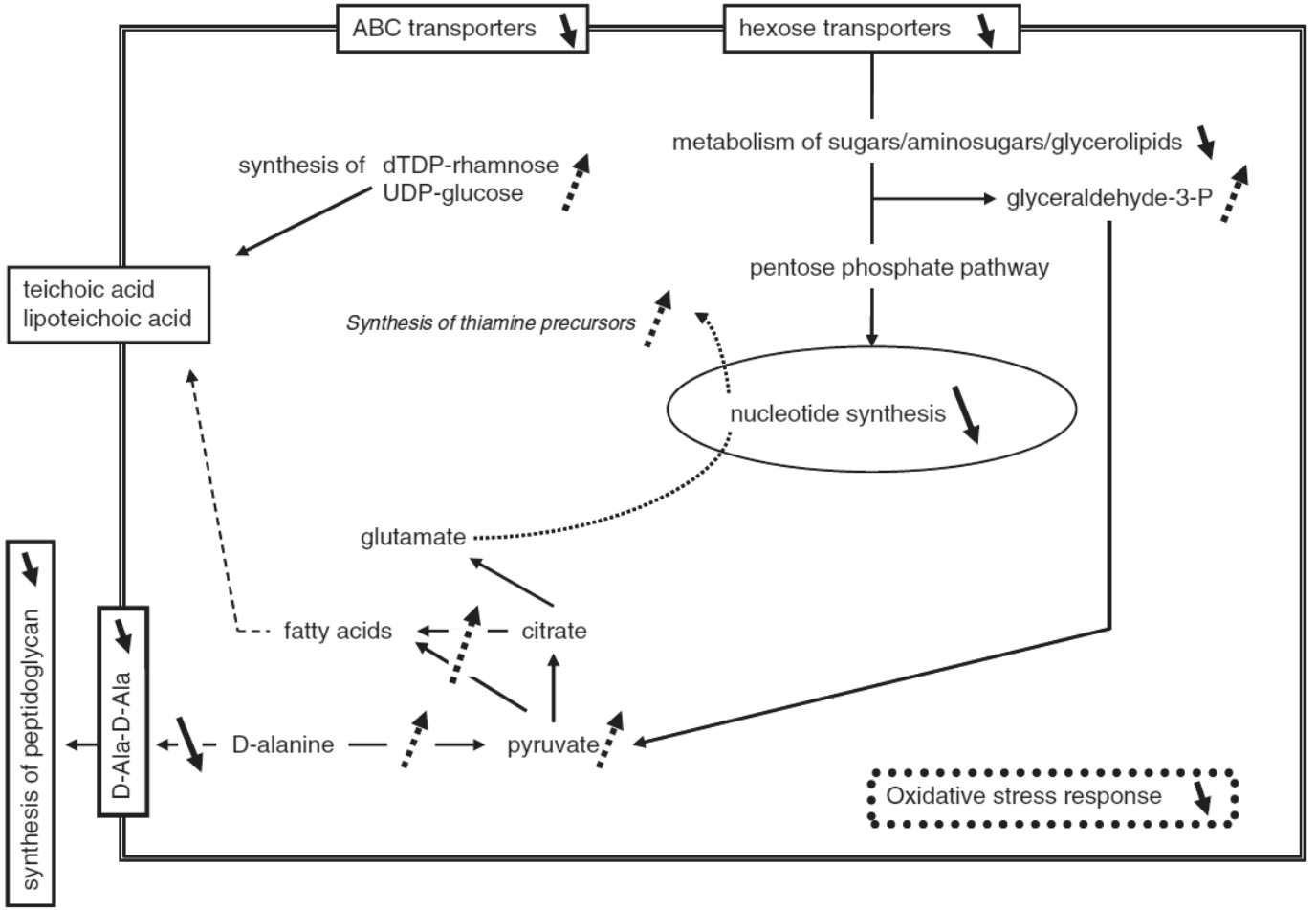
Lemaire et al. JAC (2005) 55:897–904

Bacterial responsiveness and PD

Proteomic analysis of extra- vs intra-cellular *Listeria*



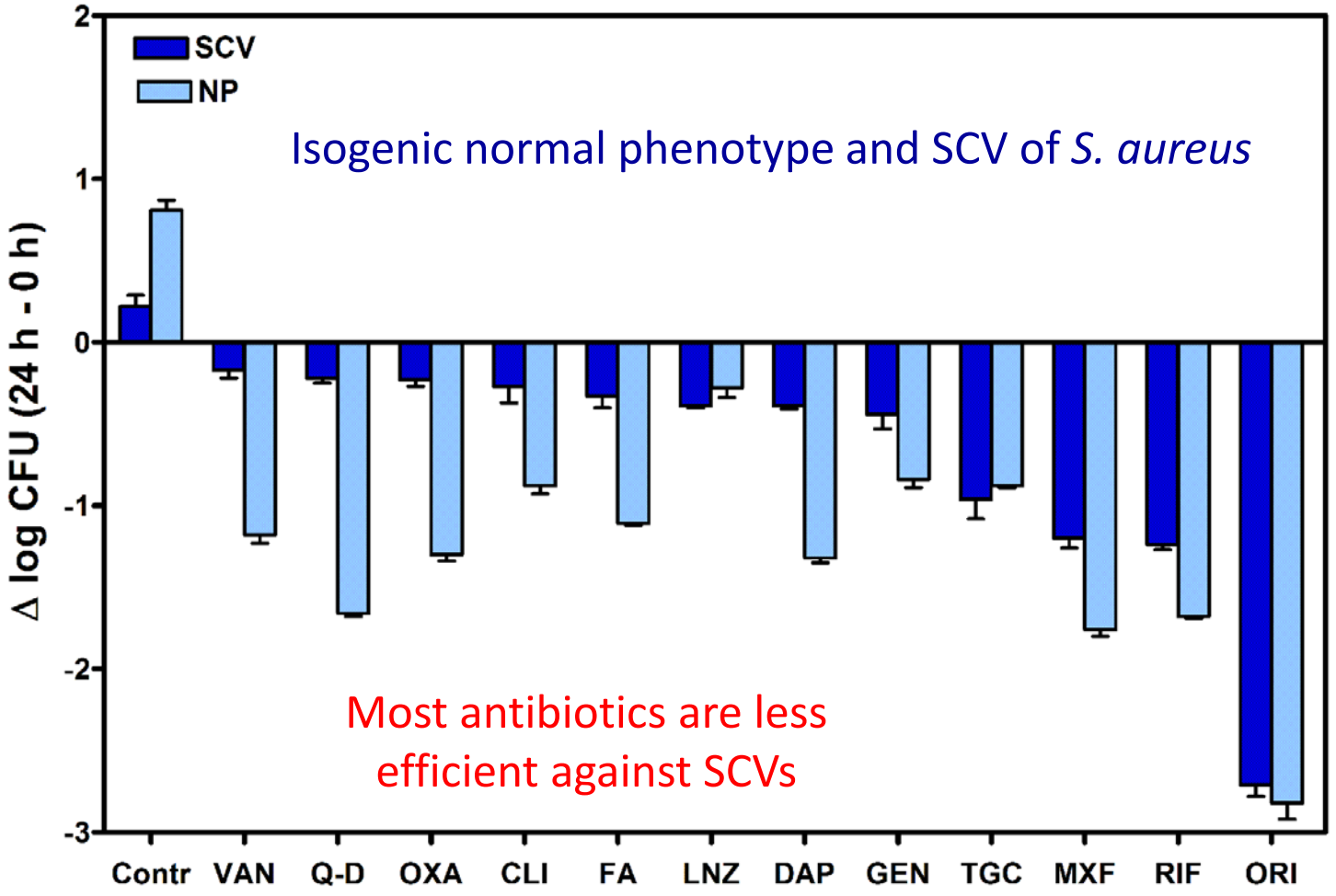
Reduced cell wall synthesis



Van de Velde et al. Proteomics (2009) 9:5484-96

Bacterial responsiveness and PD

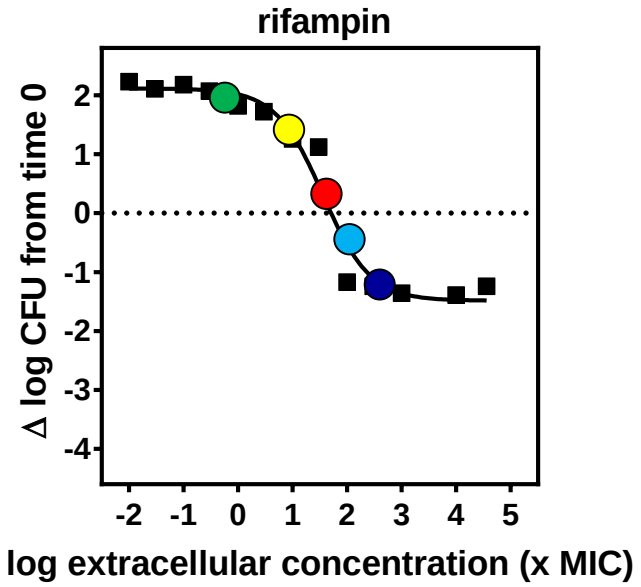
Differences among phenotypes



Nguyen et al, AAC (2009) 53:1434–42

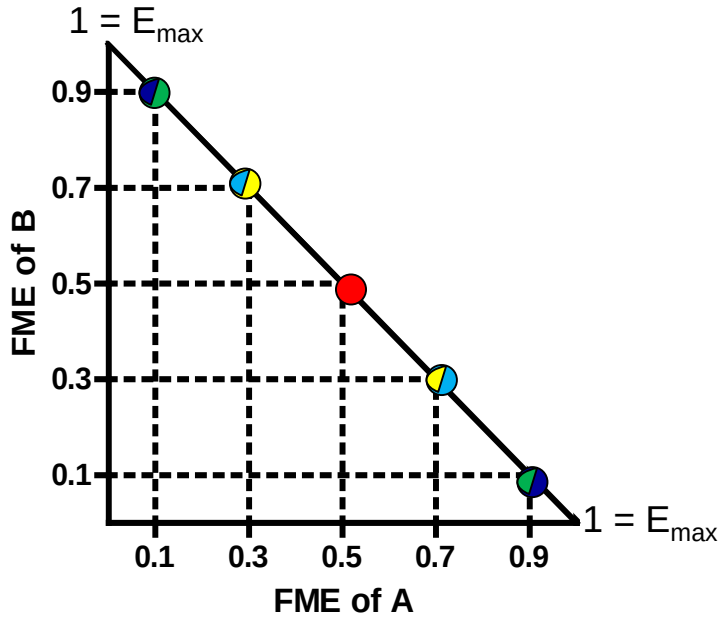
Bacterial responsiveness and PD

Combining drugs as a way to improve efficacy ?

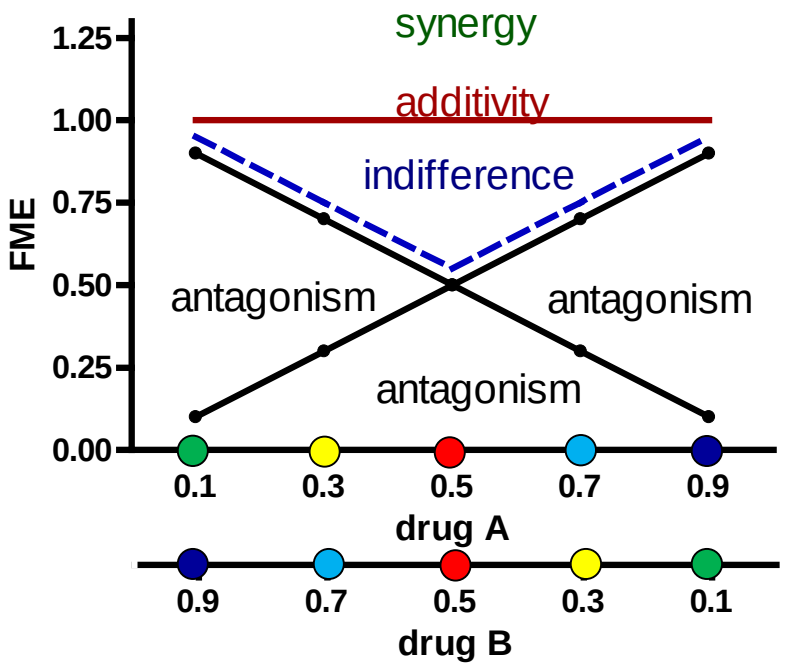


$$C_A = \frac{FME_A.EC_{50A}}{1-FME_A}$$

$$C_B = \frac{FME_B.EC_{50B}}{1-FME_B}$$



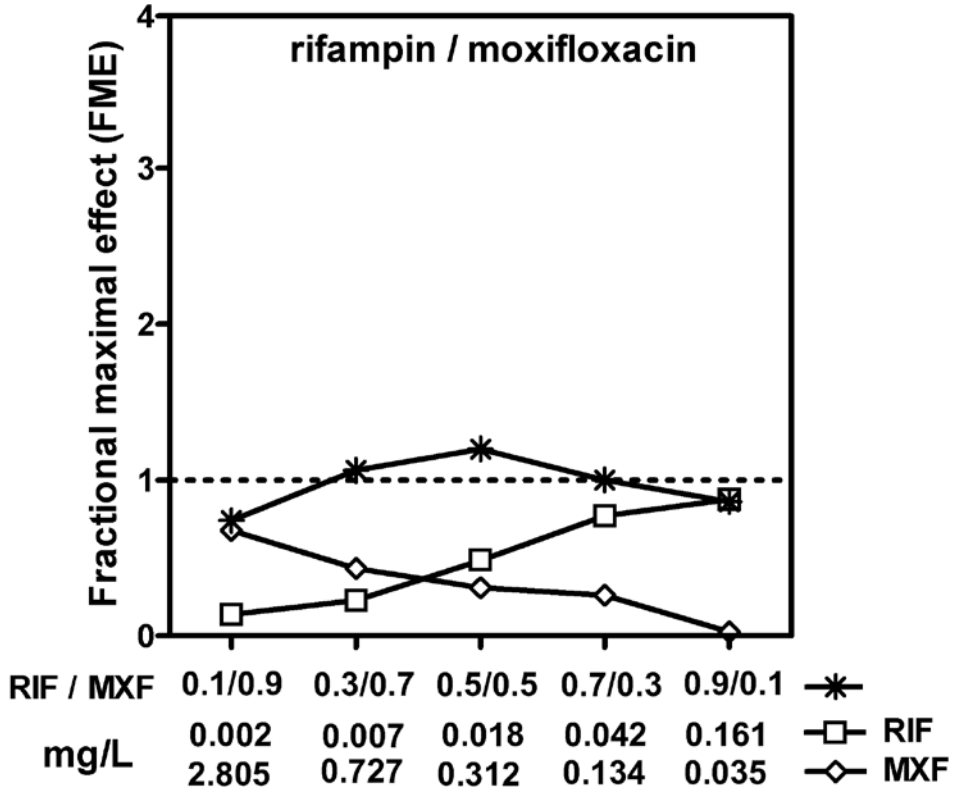
$FME_{comb.} = FME_A + FME_B = 1$

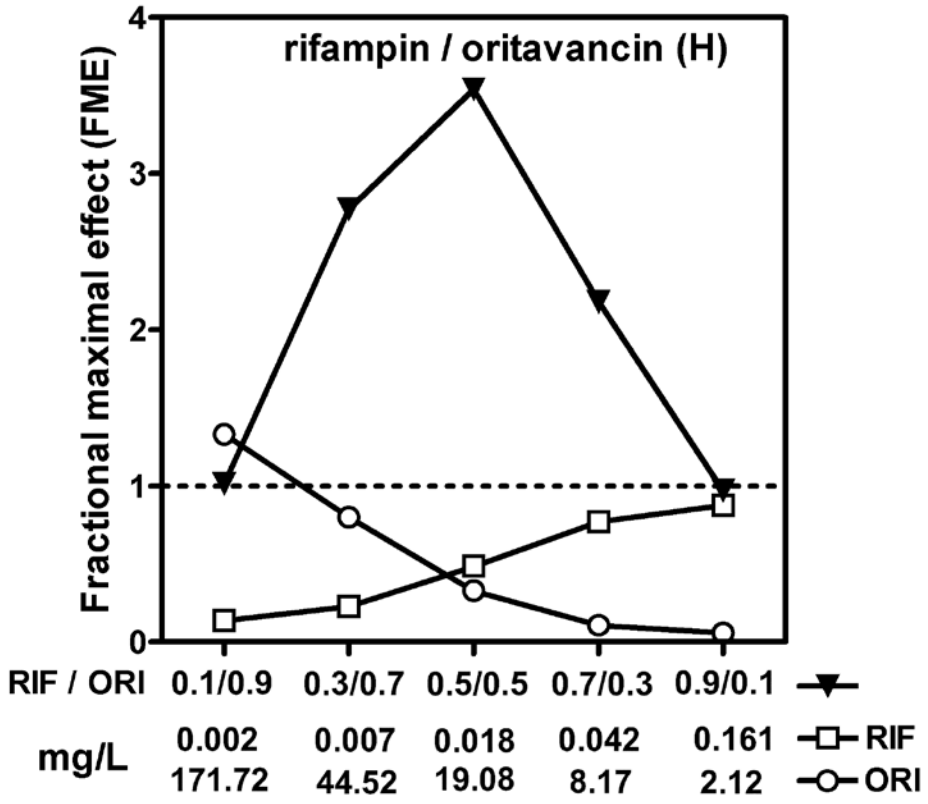


Nguyen et al, AAC (2009) 53:1443–49

Bacterial responsiveness and PD

Combining drugs as a way to improve efficacy ?

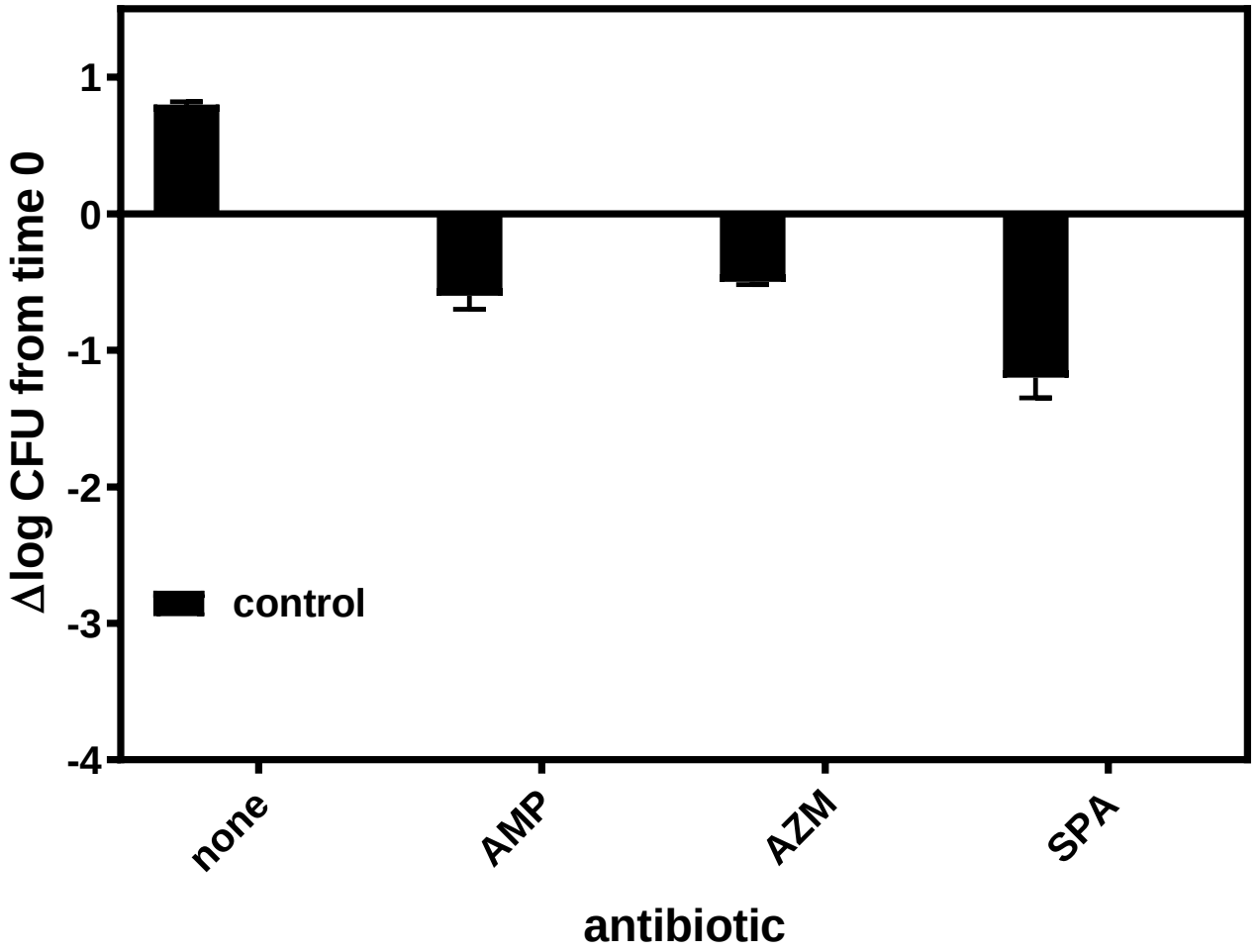




Nguyen et al, AAC (2009) 53:1443–49

Cooperation with host defenses

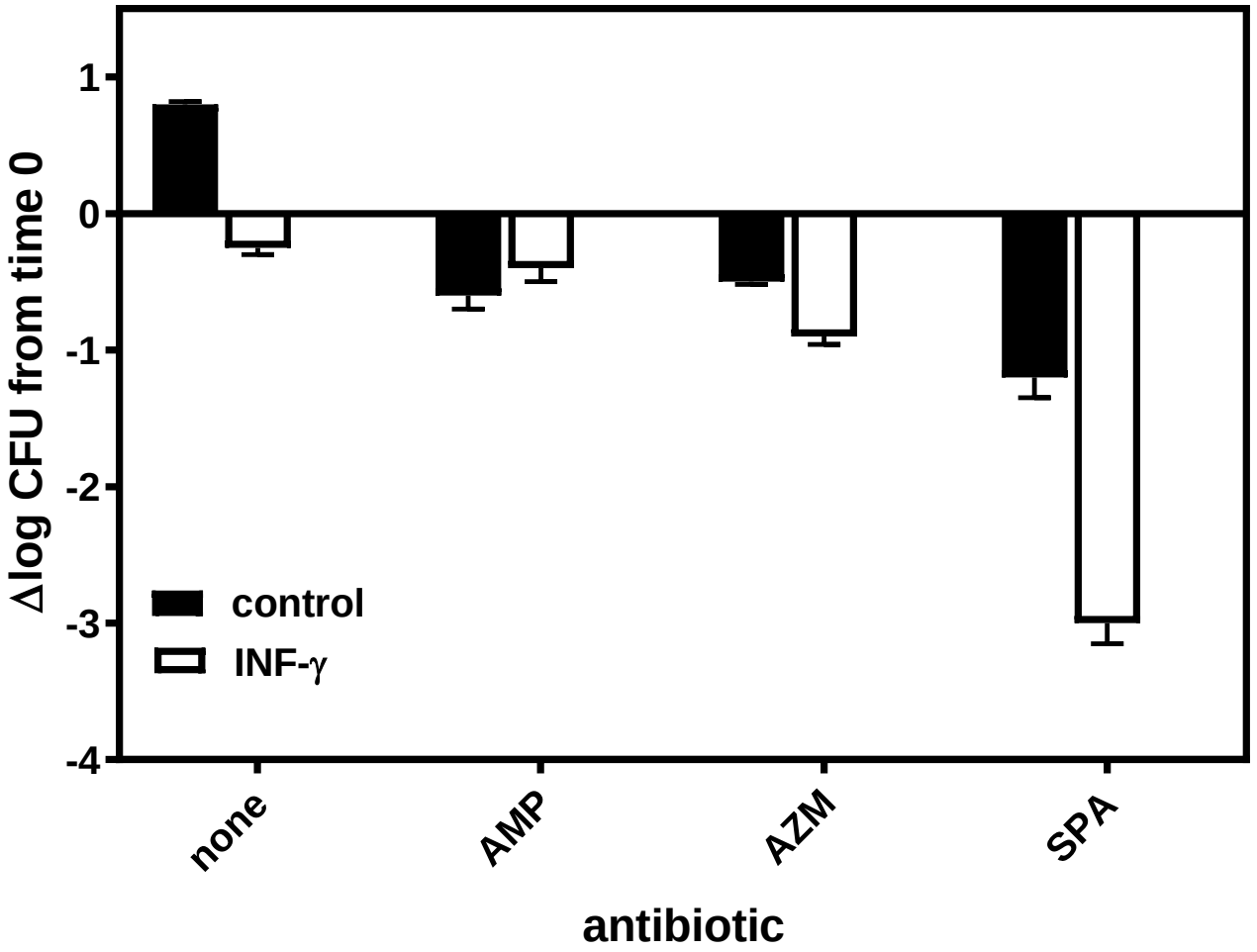
Influence of Interferon-γ on antibiotic activity towards intracellular *L. monocytogenes*



Ouadhriri et al, AAC (1999) 43:1241-51

Cooperation with host defenses

Influence of Interferon-γ on antibiotic activity towards intracellular *L. monocytogenes*



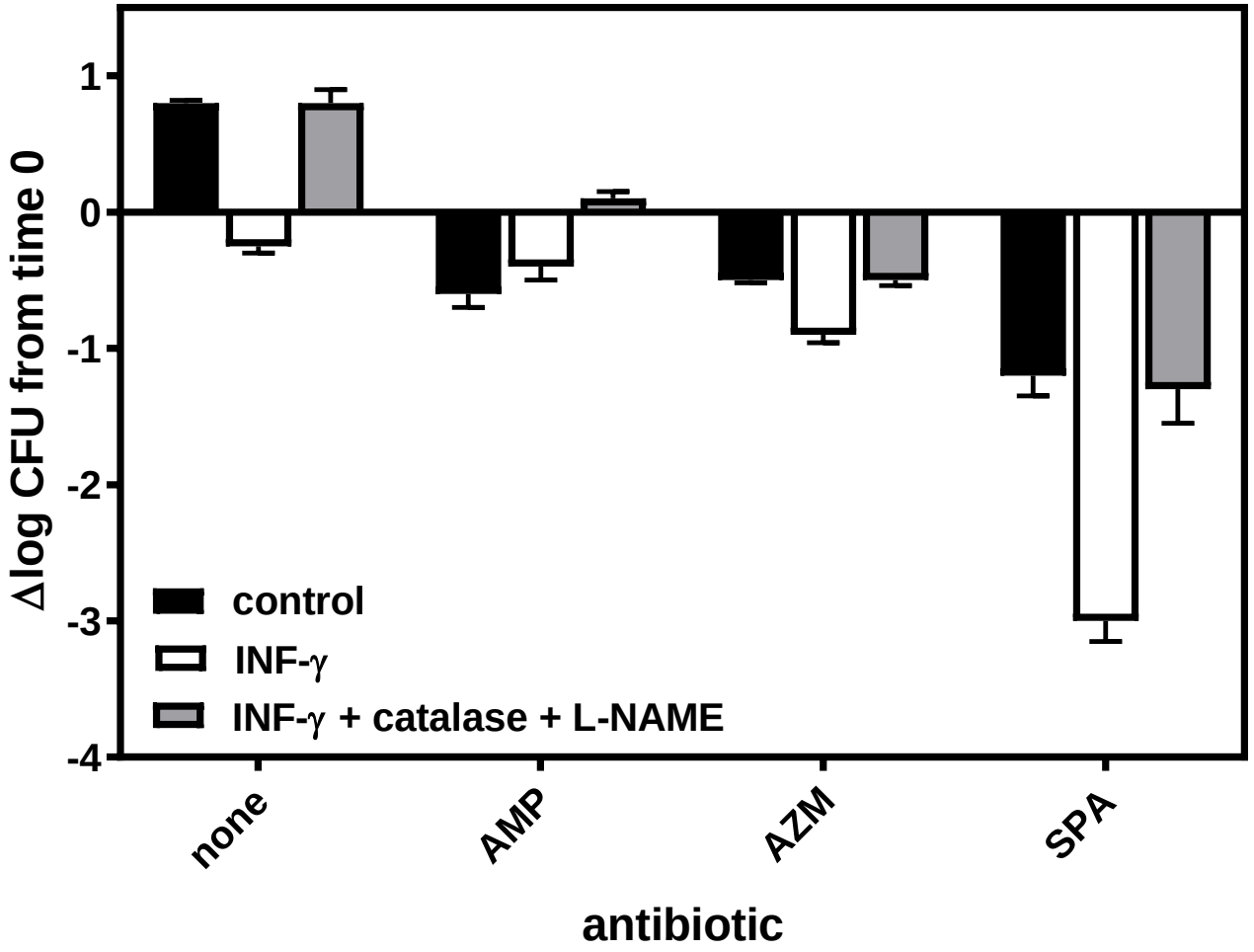
+ INF-γ:

- Bacteria confined in vacuoles
- FQ (and ML) more active: why ?

Ouadhriri et al, AAC (1999) 43:1241-51

Cooperation with host defenses

Influence of Interferon-γ on antibiotic activity towards intracellular *L. monocytogenes*



+ INF-γ and antioxidants:

- Bacteria confined in vacuoles
- AB activity ~ control conditions

Cooperation between AB and oxidant species

Ouadhriri et al, AAC (1999) 43:1241-51

Conclusion: wishlist for an antibiotic active against intracellular bacteria

PK properties

- access (and accumulation) in all subcellular compartments
- not substrate for efflux
- activity at both acidic and neutral pH
- consider risks of toxicity if prolonged retention …

PD properties

- expression of activity intracellularly, including against slow growing phenotypes
- cooperation with host defenses
- consider combinations, including with agents that can increase bacterial reponsiveness

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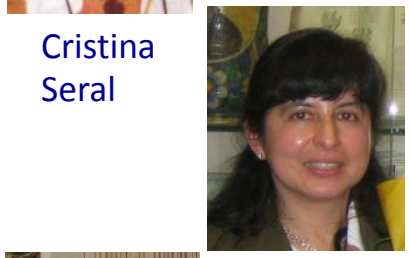

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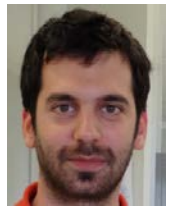
Sébastien Van de Velde

Tiep Khac Nguyen

Charlotte Misson

Marie-Claire Cambier

Katia Santos Saial



Vasileios Yfantis